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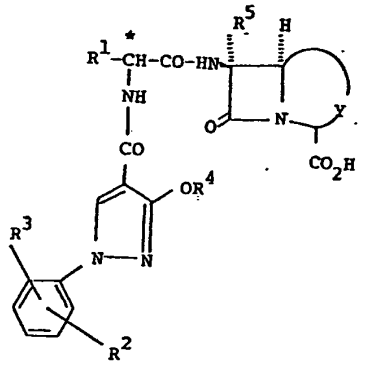

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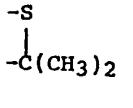

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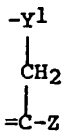

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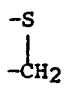

(57) Abstract

Compounds of general formula (I), their pharmaceutically acceptable salts and *in-vivo* hydrolysable esters are antibacterial agents; in which R^1 is optionally substituted phenyl or optionally substituted 5 or 6-membered heterocyclyl; R^2 is substituted amino; R^3 is H or alkyl; R^4 is H, CH_3 or CH_3COO; R^5 is H, $-OCH_3$ or -NHCO; Y is in formula (II), Y^1 is -O-, -S-, or $-CH_2-$; and Z is H, halogen or organic.

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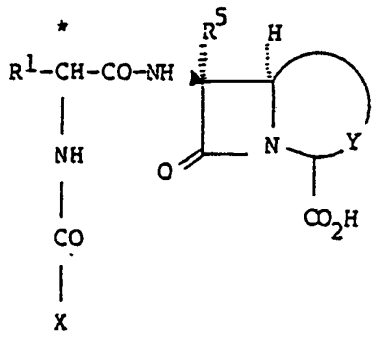
BETA-LACTAM DERIVATIVES. PREPARATION. COMPOSITIONS CONTAINING THEM

This invention relates to a class of β-lactam derivatives which have antibacterial activity and are of value in the treatment of infections in animals, especially mammals, including humans. In particular the invention relates to a class of β-lactam derivatives carrying an acylamino side-chain substituted by a substituted pyrazolyl group. The invention also relates to a process for the preparation of such compounds, and to pharmaceutical compositions containing them.

European Patent Publication No. 0 090 656 A1 describes a compound of the general formula (A) or a pharmaceutically acceptable salt or in-vivo hydrolysable ester thereof:

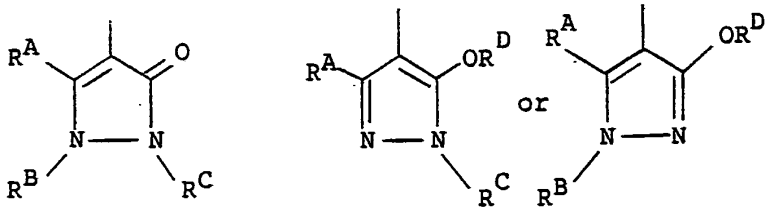


(A)

in which

R^1 denotes phenyl, substituted phenyl, or a 5- or 6-membered heterocyclic ring containing up to three heteroatoms selected from oxygen, sulphur and nitrogen, optionally substituted by hydroxy, amino, halogen or (C_{1-6})alkoxy;

X denotes:



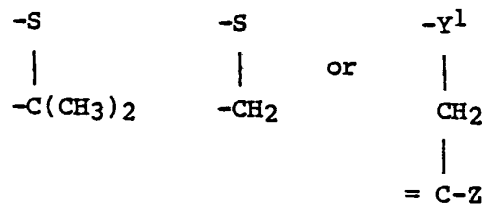
R^A and R^B may be the same or different and each denotes hydrogen, aryl, heterocyclyl, or (C_{1-6})alkyl optionally substituted by aryl or heterocyclyl;

R^C denotes hydrogen, (C_{1-6})alkylcarbonyl, aryl, heterocyclyl, or (C_{1-6})alkyl optionally substituted by aryl or heterocyclyl;

R^D denotes methyl or acetyl;

R^5 denotes hydrogen, methoxy, or -NHCHO;

Y denotes:

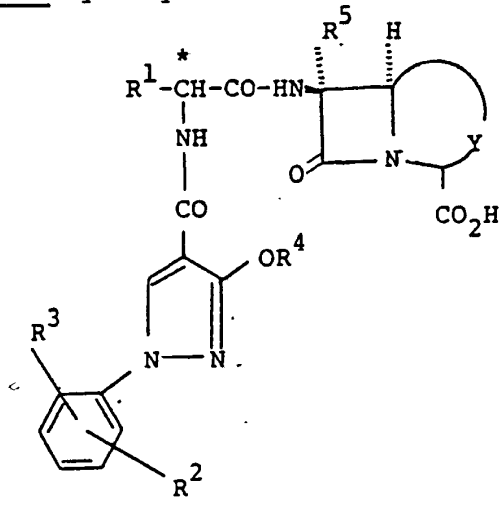


Y^1 denotes oxygen, sulphur or $-CH_2-$;

Z denotes hydrogen, halogen or an organic group such as (C_{1-4})alkoxy, $-CH_2-Q$ or $-CH=CH-Q$;

Q denotes hydrogen, halogen, hydroxy, mercapto, cyano, carboxy, carbamoyloxy, carboxylic ester, (C_{1-4})alkoxy, acyloxy, aryl, heterocyclyl bonded via carbon, heterocyclylthio, or nitrogen-containing heterocyclyl bonded via nitrogen.

The present invention provides a compound of the general formula (I) or a pharmaceutically acceptable salt or in-vivo hydrolysable ester thereof:



(I)

in which

R^1 denotes a phenyl group, a substituted phenyl group, or a 5- or 6- membered heterocyclic ring containing one, two or three heteroatoms selected from oxygen, sulphur and nitrogen, and being unsubstituted or substituted by one or more substituents selected from hydroxy, amino, halogen and (C_{1-6})alkoxy;

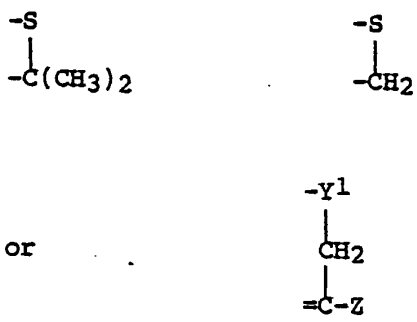
R^2 denotes a substituted amino group;

R^3 denotes hydrogen or a (C_{1-6})alkyl group;

R^4 denotes hydrogen, a methyl group, or an acetyl group;

R^5 denotes hydrogen, a methoxy group, or an -NHCHO group;

Y denotes:



Y^1 denotes an oxygen atom, a sulphur atom or a $-CH_2-$ group;

Z denotes hydrogen, a halogen atom, or an organic group, for example (C_{1-4})alkoxy, $-CH_2-Q$ or $-CH=CH-Q$;

Q denotes hydrogen, halogen, hydroxy, mercapto, cyano, carboxy, carbamoyloxy, carboxylic ester, (C_{1-4})alkoxy, acyloxy, aryl, heterocyclyl bonded via carbon, heterocyclylthio, or nitrogen-containing heterocyclyl bonded via nitrogen.

Suitably Y denotes one of the following groups:

$-S-C(CH_3)_2-$,
$-S-CH_2-$,
$-S-CH_2-C(CH_2-Q)=$, or
$-O-CH_2-C(CH_2-Q=$.

Preferred values for Y include

$-S-C(CH_3)_2-$ and
$-S-CH_2-C(CH_2-Q)=;$

that is to say, the compound of formula (I) is preferably a derivative of a penicillin or cephalosporin.

A particularly preferred value for Y is $-S-C(CH_3)_2-$.

Suitably R^5 denotes hydrogen.

Suitably R^5 denotes a methoxy group.

Suitably R^5 denotes a formamido (-NHCHO) group.

The term 'hydrocarbon' as used herein includes groups having up to 18 carbon atoms, suitably up to 10 carbon atoms, conveniently up to 6 carbon atoms. Suitable hydrocarbon groups include (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{2-6})alkynyl, (C_{3-7})cycloalkyl, (C_{3-7})cycloalkyl(C_{1-6})alkyl, aryl, and aryl(C_{1-6})alkyl.

Suitable alkyl groups include straight-chain and branched-chain alkyl groups containing from 1 to 6 carbon atoms, such as methyl, ethyl, propyl and butyl. A particular alkyl group is methyl.

Suitable optional substituents for the hydrocarbon groups, heterocyclic groups and other organic radicals include (C_{1-6})alkyl, heterocyclyl, amino, (C_{1-6})alkanoylamino, (mono, di, or tri)-(C_{1-6})alkylamino, hydroxy, (C_{1-6})alkoxy, mercapto,

(C_{1-6})alkylthio, heterocyclylthio, arylthio, aminosulphinyl, carbamoyl, amidino, guanidino, nitro, chloro, bromo, fluoro, carboxy, carboxy salts, carboxy esters, (C_{1-6})alkanoyloxy, arylcarbonyl and heterocyclylcarbonyl groups.

The term 'aryl' as used herein includes phenyl and naphthyl groups, which may be unsubstituted or substituted by up to five, preferably up to three, groups selected from halogen, (C_{1-6})alkyl, phenyl, (C_{1-6})alkoxy, halo(C_{1-6})alkyl, hydroxy, amino, nitro, aminosulphinyl, carboxy, (C_{1-6})alkoxycarbonyl, (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, (C_{1-6})alkylcarbonyloxy, and (C_{1-6})alkylcarbonyl groups.

Suitably, a substituted phenyl group R^1 is a phenyl group substituted by up to three groups selected from (C_{1-6})alkyl, phenyl, halogen, (C_{1-6})alkoxy, amino, nitro, hydroxy, (C_{1-6})alkylcarbonyloxy, carboxy, (C_{1-6})alkoxycarbonyl, halo(C_{1-6})alkyl, oxo(C_{1-6})alkyl, (C_{1-6})alkylcarbonyl, aryloxy, aralkyloxy, arylcarbonyl, (C_{1-6})alkylamino or di(C_{1-6})alkylamino.

Examples of suitable pharmaceutically acceptable *in-vivo* hydrolysable ester groups include those which breakdown readily in the human body to leave the parent acid or its salts, for example acyloxyalkyl groups, such as acetoxymethyl, pivaloyloxymethyl, α-acetoxyethyl and α-pivaloyloxyethyl groups; alkoxycarbonyloxyalkyl groups, such as ethoxycarbonyloxymethyl and α-ethoxycarbonyloxyethyl; dialkylaminoalkyl groups, such as dimethylaminomethyl, dimethylaminoethyl, diethylaminomethyl and diethylaminoethyl; and lactone groups, such as phthalidyl and dimethoxyphthalidyl.

Suitable pharmaceutically acceptable salts of the compounds of formula (I) include metal salts, for example aluminium salts, alkali metal salts, such as sodium or potassium, alkaline earth metal salts, such as calcium or magnesium, and ammonium or substituted ammonium salts, for example those with lower-alkylamines such as triethylamine; hydroxy-lower-alkylamines, such as 2-hydroxyethylamine, bis(2-hydroxyethyl)amine or tris(2-hydroxyethyl)amine; cycloalkylamines, such as bicyclohexylamine; procaine, dibenzylpiperidine, N-benzyl-β-phenethylamine, dehydroabietylamine, N,N'-bisdehydroabietylamine, ethylenediamine; or bases of the pyridine type, such as pyridine, collidine or quinoline.

The carbon atom marked * in formula (I) is asymmetric and the compound may be derived from the side-chain having a D, L or DL configuration at that position. All forms of compound (I) are included in this invention. Suitably, the carbon atom marked * is derived from the D-configuration.

Certain compounds within formula (I) may also occur in two or more tautomeric forms; all such forms are also included within the scope of the present invention.

In formula (I), the group R^1 is preferably phenyl, 4-hydroxyphenyl, 3,4-dihydroxyphenyl, 4-acetoxyphenyl, 3,4-diacetoxyphenyl, 2-thienyl, 3-thienyl or 2-amino-4-thiazolyl.

The terms 'heterocyclyl' and 'heterocyclic' as used herein include single and fused, aromatic and non-aromatic rings containing from one to four hetero-atoms in each ring selected from oxygen, nitrogen and sulphur, which rings may each suitably contain from 4

to 7, advantageously 5 or 6, ring atoms, and which rings may be unsubstituted or substituted by up to three groups selected from halogen, (C_{1-6})alkyl, (C_{1-6})alkoxy, halo(C_{1-6})alkyl, hydroxy, amino, carboxy, (C_{1-6})alkoxycarbonyl, (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, aryl, oxo, nitro, sulphonamido, (C_{1-6})alkylcarbonyl, amido, and (C_{1-6})alkylamino groups.

When used herein the term 'halogen', unless otherwise defined, suitably includes fluorine, chlorine, bromine, and iodine, preferably chlorine or bromine.

When used herein the term 'carboxylic ester' or 'carboxy ester,' unless otherwise defined, suitably includes (C_{1-6})alkyl esters.

When used herein the term 'acyloxy', unless otherwise defined, suitably includes (C_{1-6})alkylcarbonyloxy groups.

Suitable substituted amino groups R^2 include acylamino, carbamate, and ureido groups. For example, the group R^2 may represent a moiety of formula -NHCOR, -NHCO$_2$R, -NHCONHR, -NHSOR or -NHC(R)=NH; where R represents hydrogen, unsubstituted or substituted hydrocarbon, or unsubstituted or substituted heterocyclyl.

A preferred form of the present invention is when R^2 represents -NHCOR, -NHCO$_2$R or -NHCONHR.

Suitably R is hydrogen, substituted or unsubstituted (C_{1-6})alkyl, substituted or unsubstituted aryl, or a group $-OR^m$ or $-NR^pR^q$, where R^m is substituted or unsubstituted (C_{1-6})alkyl, or substituted or unsubstituted aryl, R^p is hydrogen or (C_{1-6})alkyl, and R^q is hydrogen, substituted or unsubstituted (C_{1-6})alkyl, or substituted or unsubstituted aryl.

More suitably R is hydrogen, (C_{1-6})alkyl, (C_{1-6})alkanoylamino(C_{1-6})alkyl, (C_{1-6})alkoxy, (C_{1-6})alkylamino, or phenyl which is unsubstituted or substituted by up to three groups selected from hydroxy, (C_{1-6})alkanoyloxy, and aminosulphinyl.

Particular values of R within the present invention include hydrogen, methyl, n-propyl, n-butyl, methoxy, ethoxy, phenyl, substituted phenyl wherein the substituents are selected from hydroxy, acetoxy and aminosulphinyl; benzyl; methylamino; and N-acetyl-D-alanyl.

Particular values of R^3 within the present invention include hydrogen.

Particular values of R^4 within the present invention include hydrogen.

Since the β-lactam antibiotic compounds of the present invention are intended for use in pharmaceutical compositions it will readily be understood that they are each provided in substantially pure form, for example at least 50% pure, more suitably at least 75% pure, and preferably at least 95% pure (percentages are on a weight/weight basis). Impure or less pure forms of the compounds may be used for preparing the more pure forms used in the pharmaceutical compositions. Although the purity of intermediate compounds of the present invention is less critical than is the purity of final compounds, it will readily be understood that substantially pure forms are preferred as for the β-lactam antibiotic compounds. Preferably, whenever possible, the compounds of the present invention are obtained in crystalline form.

Some of the compounds of this invention may be crystallised or recrystallised from solvents containing water. In such cases water of hydration may be formed. This invention includes within its scope stoichiometric hydrates of the compounds of the invention, as well as compounds of the invention containing variable amounts of water that may be produced by processes such as lyophilisation.

Suitable values for Q in the compounds of the formula (I) include the acetoxy group, heterocyclylthio group, and nitrogen-containing heterocyclic groups bonded via nitrogen.

The heterocyclylthio group may suitably be represented by the formula:

$$-S-Het$$

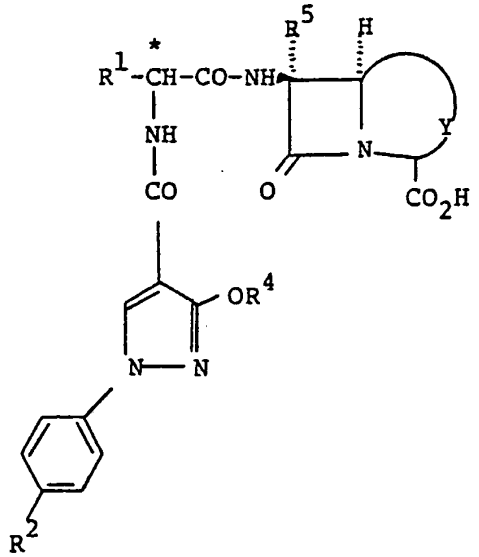
wherein 'Het' is a five- or six-membered heterocyclic ring containing from 1 to 4 hetero-atoms selected from N, O, and S and being unsubstituted or substituted by one or two groups selected from (C_{1-6})alkyl, (C_{1-6})alkoxy, hydroxyalkyl, (C_{1-6})alkenyl, alkoxyalkyl, carboxyalkyl, sulphonylalkyl, carbamoylalkyl, trifluoromethyl, hydroxy, halogen, oxo, aminoalkyl, substituted-aminoalkyl, and carboxyalkyl, or beingfused to a second heterocyclic ring or a carbocyclic ring.

Examples of the group 'Het' include unsubstituted and substituted imidazolyl, triazolyl, tetrazolyl, thiazolyl, thiadiazolyl, thiatriazolyl, oxazolyl, triazinyl, and oxadiazolyl.

Suitable groups 'Het' include unsubstitut d and substituted 1,2,3-triazolyl; 1,2,4-triazolyl; tetrazolyl; oxazolyl; thiazolyl; 1,3,4-oxadiazolyl; 1,3,4-thiadiazolyl; and 1,2,4-thiadiazolyl. Preferably the heterocyclylthio group is 1-methyl-1H-tetrazol-5-ylthio, 2-methyl-1,3,4-thiadiazol-5-ylthio, 1-carboxymethyl-1H-tetrazol-5-ylthio, or 6-hydroxy-2-methyl-5-oxo-2H-1,2,4-triazin-3-ylthio.

The nitrogen-containing heterocyclic group bonded via nitrogen is suitably an unsubstituted or substituted pyridinium group. Suitably the pyridinium group is substituted by one or two groups selected from (C_{1-6})alkyl, (C_{1-6})alkoxy, hydroxyalkyl, (C_{1-6})alkenyl, alkoxyalkyl, carboxyalkyl, sulphonylalkyl, carbamoylmethyl, carbamoyl, trifluoromethyl, hydroxy, halogen, oxo, and aminoalkyl.

One preferred group of compounds within the present invention are the compounds of formula (II) and their pharmaceutically acceptable salts and in vivo hydrolysable esters:



(II)

wherein R^1, R^2, R^4, R^5, Y and * have the meanings given above.

Specific compounds within this invention include the following compounds and pharmaceutically acceptable salts and in-vivo hydrolysable esters thereof:

6-β-[D,2-(2-p-acetylaminophenyl-3-pyrazolin-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid;

6β-[D,2-(2-p-acetylaminophenyl-3-pyrazolin-5-one-4-carbonylamino)-2-(4-hydroxyphenyl)]acetamido penicillanic acid;

6β-[D,2-([1,H]-1-[4-n-butyramido]phenyl-3-methoxy pyrazole-4-carbonylamino)-2-phenyl]acetamido penicillanic acid;

6β-[D,2-(2-(4-benzamidophenyl)pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid;

6β-[D,2-(2-(4-benzamidophenyl)pyrazol-3-in-5-one-4-carbonylamino)-2-(4-hydroxyphenyl)] acetamido penicillanic acid;

6β-[D,2-(2-p-n-butyramidophenyl pyrazol-3-in-5-one-4--carbonylamino)-2-phenyl]acetamido penicillanic acid;

6β-[D,2-(2-p-n-butyramidophenyl pyrazol-3-in-5-one-4-carbonylamino)-2-(4-hydroxyphenyl)]acetamido penicillanic acid;

6β-[D,2-(2-[4-(3,4-diacetoxybenzamido)phenyl]-3-pyrazolin-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid;

6β-[D,2-([1,H]-3-acetoxy-1-[4-benzamido phenyl]-pyrazole-4-carbonyl amino)-2-(3,4-diacetoxyphenyl)] acetamido penicillanic acid;

6β-[D,2-(2-[4-benzamidophenyl]pyrazol-3-in-5-one-4-carbonylamino)-2-(3,4-diacetoxyphenyl)] acetamido penicillanic acid;

6β-[D,2-([1,H]-3-acetoxy-1-[4-n-butyramidophenyl]-pyrazole-4-carbonylamino)-2-(3,4-diacetoxyphenyl)] acetamido penicillanic acid;

6β-[D,2-(2-(4-n-butyramidophenyl)pyrazol-3-in-5-one-4-carbonylamino)-2-(3,4-diacetoxyphenyl)] acetamido penicillanic acid;

6β-[D,2-[2-(4-benzoylaminophenyl)-3-pyrazolin-5-one-4-carbonylamino]-2-phenyl]acetamido-6,α-formamido penicillanic acid;

6β-[D,2-([1,H]-3-acetoxy-1-[4-benzoylaminophenyl]-pyrazole-4-carbonylamino)-2-(4-hydroxyphenyl)]acetamido -6α-formamido penicillanic acid;

6β-[D,2-([1,H]-3-acetoxy-1-(4-n-butyramidophenyl)-pyrazole-4-carbonylamino)-2-(3,4-diacetoxyphenyl)]-acetamido bisnorpenicillanic acid;

6β-[D,2-(2-p-methylsulphonylaminophenyl pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid;

6β-[D,2-(2-p-methoxycarbonylaminophenyl pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid;

6β-[D,2-(2-(4-N-acetyl-D-alanylamino)phenyl pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid;

6β-[D,2-(2-(4-ethoxycarbonylaminophenyl) pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid;

6β-[D,2-(2-[4-phenylmethylcarbonylaminophenyl] pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid;

6β-[D,2-(2-[4-methylaminocarbonylaminophenyl] pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid;

6β-[D,2-phenyl-2-(2-(4-n-proprionamidophenyl) pyrazol-3-in-5-one-4-carbonylamino)]acetamido penicillanic acid;

6β-[D,2-(2-[4-(3,5-dihydroxybenzamido)phenyl]-pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid;

6β-[D,2-phenyl-2-(2-[4-(2,4,6-triacetoxybenzamido)-phenyl]-pyrazol-3-in-5-one-4-carbonylamino)]acetamido penicillanic acid;

6β-[D,2-(2-[4-formamido phenyl]-pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid;

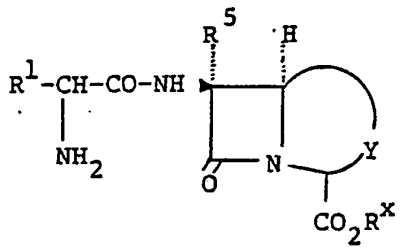
6β-[D,2-(4-hydroxyphenyl)-2-(2-[4-methoxycarbonyl-amino phenyl] pyrazol-3-in-5-one-4-carbonylamino)] acetamido penicillanic acid;

6β-[D,2-(2-[4-(4-aminosulphonyl benzamido)phenyl] pyrazole-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid;

6β-[D,2-(2-[4-(3,4-dihydroxybenzamido)phenyl]pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid;

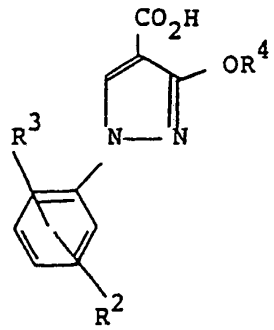
6β-[D,2-(2-(3-methoxycarbonylaminophenyl)pyrazole-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid.

The compounds of the invention may be prepared by reacting a compound of formula (III):



(III)

wherein the amino group is optionally substituted with a group which permits acylation to take place, R^1, R^5 and Y are as defined with respect to formula (I), any reactive groups may be protected, and R^x is hydrogen or a carboxyl-blocking group, with an N-acylating derivative of a compound of formula (IV):



(IV)

wherein R^2, R^3 and R^4 are as hereinbefore defined and wherein any reactive groups may be protected; and thereafter, if necessary, carrying out one or more of the following steps:

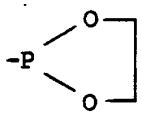
i) removing any carboxyl-blocking group R^x;

ii) removing any protecting groups on the side-chain group;

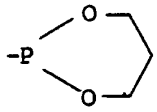
iii) converting one group Z to a different group Z;

iv) converting the product into a salt or in-vivo hydrolysable ester thereof.

Suitable groups which permit acylation to take place and which are optionally present on the amino group of the starting material of the formula (III) include N-silyl groups, for example trialkylsilyl groups, such as trimethylsilyl; groups of formula $-PR^aR^b$, wherein R^a is an alkyl, haloalkyl, aryl, aralkyl, alkoxy, haloalkoxy, aryloxy, aralkyloxy or dialkylamino group, R^b is the same as R^a or is halogen, or R^a and R^b together denote a ring; suitable such phosphorus groups being $-P(OC_2H_5)_2$, $-P(C_2H_5)_2$,



and



Suitable carboxyl-blocking derivatives for the group $-CO_2R^x$ in formula (III) include salts and ester derivatives of the carboxylic acid. The derivative is preferably one which may readily be cleaved at a later

stage of the reaction. Suitable salts include metal salts, such as those with sodium, potassium and lithium, and tertiary ammonium salts, such as those with trilower-alkylamines, N-ethylpiperidine, 2,6-lutidine, pyridine, N-methylpyrrolidine, dimethylpiperazine. A preferred salt is the triethylammonium salt.

Suitable ester-forming carboxyl-blocking groups are those which may be removed under conventional conditions. Such groups for R^x include benzyl, p-methoxybenzyl, 2,4,6-trimethylbenzyl, 3,5-di-t-butyl-4-hydroxy-benzyl, benzoylmethyl, p-nitrobenzyl, 4-pyridylmethyl, 2,2,2-trichloroethyl, 2,2,2-tribromoethyl, diphenylmethyl, triphenylmethyl, adamantyl, 2-benzyloxyphenyl, 4-methylthiophenyl, tetrahydrofur-2-yl, tetrahydropyran-2-yl, pentachlorophenyl, allyl, p-toluenesulphonylethyl, methoxymethyl, a silyl or phosphorus-containing group, such as described above, an oxime radical of formula $-N=CHR^o$ where R^o is aryl or heterocyclyl, or an in-vivo hydrolysable ester radical such as defined above.

The carboxyl group may be regenerated from any of the above esters by usual methods appropriate to the particular R^x group, for example, acid-catalysed, base-catalysed or enzymically-catalysed hydrolysis, or hydrogenation.

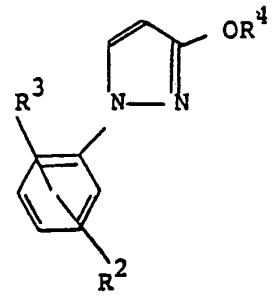
A reactive N-acylating derivative of the compound of formula IV is employed in the above process. The choice of reactive derivative will of course be influenced by the chemical nature of the substituents of the acid.

Suitable N-acylating derivatives include an acid halide, preferably the acid chloride or bromide.

Acylation with an acid halide may be effected in the presence of an acid binding agent, for example a tertiary amine (such as triethylamine or dimethylaniline), an inorganic base (such as calcium carbonate or sodium bicarbonate), or an oxirane, which binds hydrogen halide liberated in the acylation reaction. The oxirane is preferably a 1,2-(C_{1-6})alkylene oxide, for example ethylene oxide or propylene oxide. The acylation reaction using an acid halide may be carried out at a temperature in the range of from -50°C to +50°C, preferably -20°C to +20°C, in aqueous or non-aqueous media, for example aqueous acetone, aqueous tetrahydrofuran, ethyl acetate, dimethylacetamide, dimethylformamide, acetonitrile, dichloromethane, 1,2-dichloroethane, or mixtures thereof. Alternatively, the reaction may be carried out in an unstable emulsion of water-immiscible solvent, especially an aliphatic ester or ketone, such as methyl isobutyl ketone or butyl acetate.

The acid halide may be prepared by reacting a compound of formula (IV) or a salt thereof with a halogenating (eg chlorinating or brominating) agent such as phosphorus pentachloride, thionyl chloride or oxalyl chloride.

The acid chloride may also be prepared by reacting a compound of formula (IVA):



(IVA)

wherein R^2, R^3 and R^4 are as hereinbefore defined and wherein any reactive groups may be protected, with phosgene.

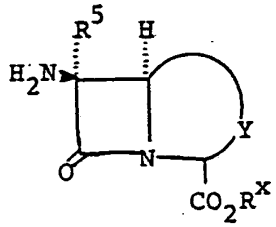
Another suitable N-acylating derivative of the compound of formula (IV) is a symmetrical or mixed anhydride. Suitable mixed anhydrides are alkoxyformic anhydrides, or anhydrides with, for example, carbonic acid monoesters, trimethyl acetic acid, thioacetic acid, diphenylacetic acid, benzoic acid, phosphorus acids (such as phosphoric or phosphorous acids) or aliphatic or aromatic sulphonic acids (such as p-toluenesulphonic acid). When a symmetrical anhydride is employed, the reaction may be carried out in the presence of 2,6-lutidine as catalyst.

Further suitable N-acylating derivatives of the compound of formula (IV) are the acid azide; activated esters, such as esters with 2-mercaptopyridine, cyanomethanol, p-nitrophenol, 2,4-dinitrophenol, thiophenol, halophenols, including pentachlorophenol, monomethoxyphenol, N-hydroxy succinimide, or 8-hydroxyquinoline; amides, such as N-acylsaccharins or N-acylphthalimides; and alkylidene iminoesters prepared by reaction of the acid $X\text{-}CO_2H$ with an oxime.

Other reactive N-acylating derivatives of the acid X-CO_2H include the reactive intermediates formed by reaction in situ with a condensing agent, such as a carbodiimide, for example, N,N-diethyl-, N,N-dipropyl- or N,N-diisopropyl-carbodiimide, N,N'-di-cyclohexyl-carbodiimide, or N-ethyl-N'-dimethylaminopropyl-carbodiimide; a suitable carbonyl compound, for example, N,N'-carbonyldiimidazole or N,N'-carbonyldi-triazole; an isoxazolinium salt, for example, N-ethyl-5-phenylisoxazolinium-3-sulphonate or N-t-butyl-5-methylisoxazolinium perchlorate; or an N-alkoxycarbonyl 2-alkoxy-1,2-dihydroquinoline, such as N-ethoxycarbonyl 2-ethoxy-1,2-dihydroquinoline. Other condensing agents include Lewis acids (for example BBr_3 - C_6H_6); or a phosphoric acid condensing agent, such as diethylphosphorylcyanide. The condensation reaction is preferably carried out in an organic reaction medium, for example, methylene chloride, dimethylformamide, acetonitrile, alcohol, benzene, dioxan or tetrahydrofuran.

The compound of formula (IV) and N-acylating derivatives thereof are novel compounds and form a further aspect of the present invention.

The intermediate compound of formula (III) may be prepared by reacting a compound of formula (V):



(V)

wherein the amino group is optionally substituted with a group which permits acylation to take place and R^5, R^x and Y are as defined with respect to formula (I) above,

with an N-acylating derivative of an acid of formula (VI):

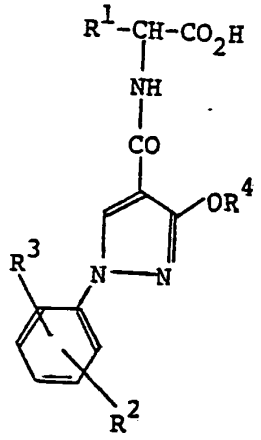
$$R^1\text{-}CH(NHR^Y)\text{-}CO_2H \qquad \text{(VI)}$$

wherein R^1 is as defined with respect to formula (I) and any reactive groups therein may be protected and R^Y is an amino-protecting group; and thereafter removing protecting group R^Y.

Suitable N-acylating derivatives, carboxyl protecting groups and reaction conditions include those described hereinbefore.

Suitable amino-protecting groups R^Y are those well known in the art which may be removed under conventional conditions without disruption of the remainder of the molecule.

The compounds of formula (I) may also be prepared by reacting a compound of formula (V) as described hereinbefore with an N-acylating derivative of an acid of formula (VII):



(VII)

wherein R^1, R^2, R^3 and R^4 are as defined with respect to formula (I) and any reactive groups therein may be protected; and thereafter, if necessary, carrying out one or more of the following steps:

i) removing any carboxyl-blocking group R^x;

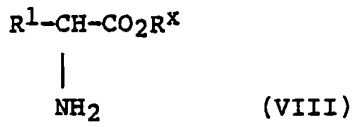
ii) removing any protecting groups on the side-chain group;

iii) converting one group Z to a different group Z;

iv) converting the product into a salt or in-vivo hydrolysable ester.

The acid (VII) and N-acylating derivatives thereof are novel compounds and form a further aspect of the present invention.

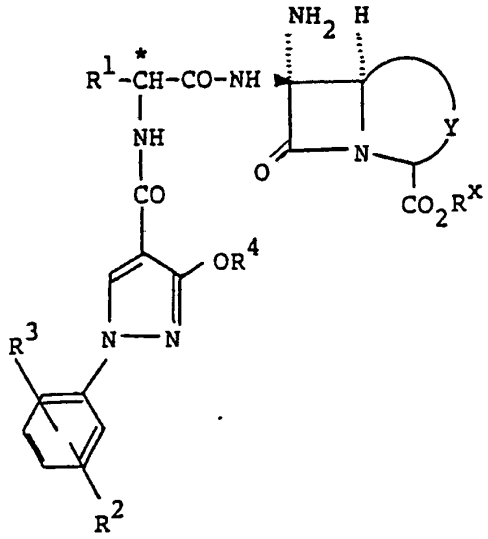
The acid (VII) may be prepared by reacting an amino-acid of formula (VIII):



(VIII)

wherein the amino group is optionally substituted with a group which permits acylation to take place, and R^1 and R^x are as defined hereinbefore, with an N-acylating derivative of a compound of formula IV as hereinbefore defined.

The present invention further provides a process for the preparation of a compound of formula (I) wherein R^5 is -NHCHO which process comprises formylating a compound of formula (IX):



(IX)

wherein R^1, R^2, R^3, R^4, R^x, Y and * have the meanings given hereinbefore and any reactive groups may be protected; and thereafter, if necessary, carrying out one or more of the following steps:

i) removing any carboxyl-blocking group R^x;

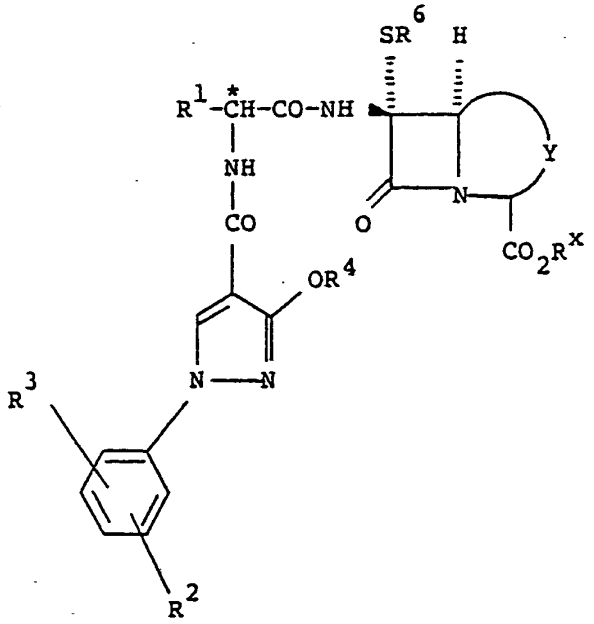
ii) removing any protecting groups on the side chain group;

iii) converting one group Z to a different group Z;

iv) converting the product into a salt or in-vivo hydroyable ester thereof.

Suitable formylating agents include mixed anhydrides such as formic acetic anhydride. The reaction may suitably be carried out in a temperature in the range of from -50°C to +30°C in an aprotic solvent, such as, for example, dichloromethane, chloroform, dimethylformamide, tetrahydrofuran, hexamethylphosphoramide, or dimethylsulphoxide, in the presence of a tertiary base. A preferred tertiary base for use in the reaction is a base of the pyridine type, such as pyridine, lutidine or picoline.

Compounds of the formula (IX) may be prepared by the reaction of a corresponding compound of the formula (X):



(X)

wherein Y, R^1, R^2, R^3, R^4, and R^x are as hereinbefore defined, and R^6 is (C_{1-6})alkyl, aryl or benzyl; with anhydrous ammonia, an ammonium salt or an amine of the formula (XI):

$$R^7\text{-}NH_2 \qquad (XI)$$

wherein R^7 is a removable protecting group such as benzyl; in the presence of a metal ion such as mercury, silver, thallium, lead or copper and thereafter if necessary removing any protecting group to form the compound of formula (X).

Suitable examples of the alkyl group R^6 include (C_{1-6})alkyl groups, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, and tert-butyl groups. A preferred alkyl group R^6 is methyl.

Suitable examples of the aryl group R^6 include phenyl, optionally substituted by (C_{1-6})alkyl, (C_{1-6})alkoxy, halogen, or nitro. Preferred aryl groups R^6 include phenyl, (o, m or p)-methylphenyl, and (o, m or p)-nitrophenyl, especially p-methylphenyl.

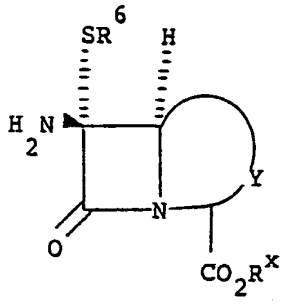
Suitable solvents in which the reaction may be performed include for example, diethyl ether, tetrahydrofuran, dimethylformamide, methanol and hexamethylphosphoramide. The reactions are generally carried out under an inert atmosphere and at moderate to low temperatures, suitably in the range of from -100°C to +30°C. The course of the reaction may be followed by conventional methods such as thin-layer chromatography and terminated when an optimum quantity of product is present in the reaction mixture.

The preferred metal ion for use in the above process is the mercuric ion, aptly in the form of mercuric acetate.

The intermediate compound of formula (X) may suitably be converted to a compound of formula (I) wherein R^5 is methoxy by reaction with methanol in the presence of a metal ion such as mercury, silver, aluminium, lead or copper under conditions analogous to those described hereinbefore for the preparation of a compound of formula (IX).

It will be appreciated that the processes for preparation of a compound of formula (IX), and also processes for the preparation of a compound of formula (I) wherein R^5 is methoxy, described hereinbefore proceed <u>via</u> an imine intermediate; other processes proceeding <u>via</u> such an intermediate are also included herein.

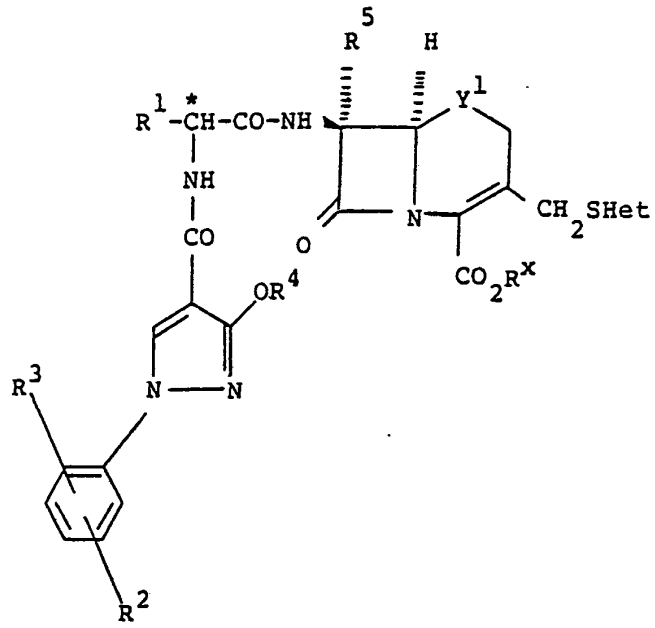
The intermediate compound of formula (X) is suitably prepared by acylation of the compound of formula (XII):



(XII)

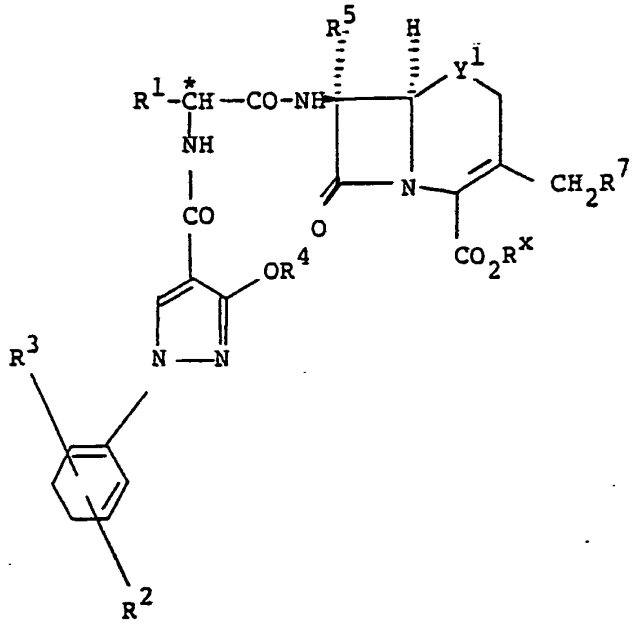
in which R^6, R^x and Y are as hereinbefore defined, with an acid of formula VII as hereinbefore defined.

The sub-group of compounds within the present invention of formula (XIII):



(XIII)

wherein Y^1, Het, R^1, R^2, R^3, R^4, R^5 and R^x as defined hereinbefore may suitably be prepared by reacting a compound of formula (XIV):



(XIV)

wherein Y^1, R^1, R^2, R^3, R^4, R^5 and R^x are as defined hereinbefore and wherein any reactive groups may be protected and R^7 is a leaving group; with a thiol of formula:

HetSH

with the proviso that when R^7 is an acyloxy group $-CO_2R^x$ must be in the free acid form or a salt thereof.

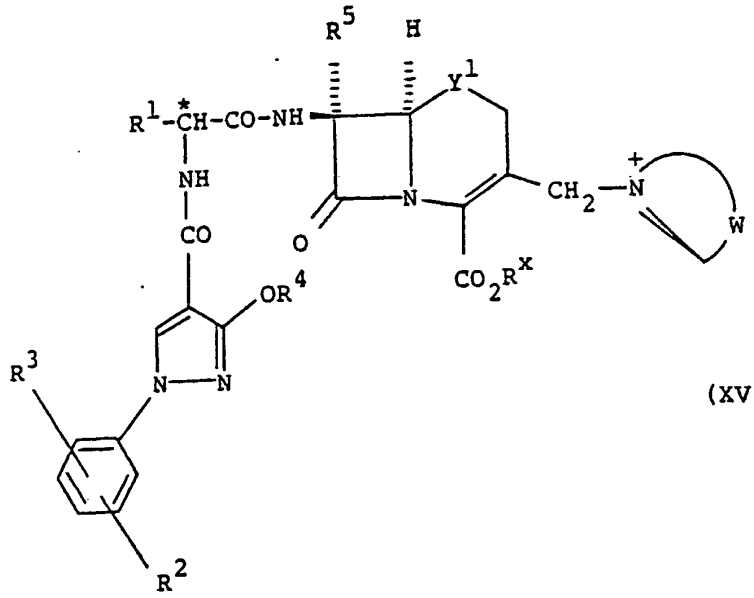
Suitable leaving groups R^7 include halogen, such as iodide or bromide, and acyloxy groups, such as, for example, the acetyloxy group.

The thiol HetSH may be reacted as the free compound or as a salt with an alkali metal, such as sodium or potassium. This reaction is desirably conducted in a solvent. For example, use can be made of water, or

organic solvents inert to the starting compounds, such as dimethylformamide, dimethylacetamide, dioxane, acetone, alcohol, 1,2-dichloroethane, acetonitrile, dimethylsulfoxide or tetrahydrofuran, or mixtures thereof. The reaction temperature and time depend, among other factors, upon the starting compounds and solvent to be employed but generally the reaction is carried out at a selected temperature within the range of from 0°C to 100°C for a selected time of a few hours to several days. The reaction is desirably conducted between pH 3 and 7.

To prevent oxidation of the thio compounds it is advantageous to carry out the reaction in an inert gaseous atmosphere, eg nitrogen gas.

The subgroup of compounds within the present invention of formula (XV):



(XV)

wherein W represents the residue of a pyridinium group unsubstituted or substituted by one or two groups selected from (C_{1-6})alkyl, (C_{1-6})alkoxy, hydroxyalkyl, (C_{1-6})alkenyl, alkoxyalkyl, carboxyalkyl, sulphonylalkyl, carbamoylmethyl, carbamoyl, trifluroromethyl, hydroxy, halogen, oxo, and aminoalkyl; and R^1, R^2, R^3, R^4, R^5, R^x and Y^1 are as defined hereinbefore; may suitably be prepared by reacting a compound of formula (XIV) as hereinbefore defined with the appropriately substituted pyridine.

The antibiotic compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other antibiotics, and the invention therefore includes within its scope a pharmaceutical composition comprising a compound of formula (I) above together with a pharmaceutical carrier or excipient.

The composition may be formulated for administration by any route, such as oral, topical or parenteral. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

Tablets and capsules for administration may be in unit-dose presentation form, and may contain conventional excipients, such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; and acceptable wetting agents, such as sodium lauryl

sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring and colouring agents.

Suppositories contain conventional suppository bases, e.g. coca-butter or other glyceride.

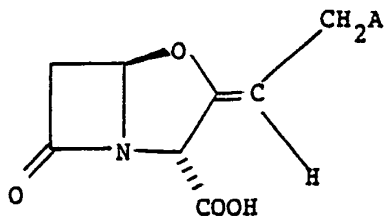
For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing. Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of

water for injection may be supplied to reconstitute the liquid prior to use. Parental suspensions are prepared in substanially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The compound can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% by weight, preferably from 10 to 60% by weight, of the active material, depending on the method of administration. Where the composition comprises dosage units, each unit will preferably contain from 50 to 500 mg of the active ingredient. The dosage as employed for adult human treatment will preferably range from 100 to 3000 mg per day, for instance 1500 mg per day depending on the route and frequency of administration. Such a dosage corresponds to 1.5 to 50 mg/kg per day. Suitably the dosage is from 5 to 20 mg/kg per day.

The compound of formula (I) may be the sole therapeutic agent in the compositions of the invention or a combination with other antibiotics and/or with a β-lactamase inhibitor may be employed.

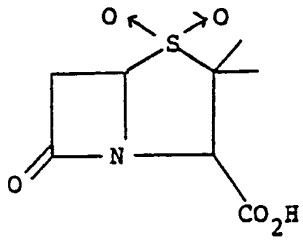
Advantageously, the compositions also comprise a β-lactamase inhibitor of formula (XVI) or a pharmaceutically acceptable salt or ester thereof:



(XVI)

wherein A is hydroxyl, substituted hydroxyl, thiol, substituted thiol, amino, (mono or di)-hydrocarbyl-substituted amino, or (mono or di)-acylamino.

A further advantageous composition comprises a compound of formula (I) or a pharmaceutically acceptable salt or in-vivo hydrolysable ester thereof together with a β-lactamase inhibitor of formula (XVII) or a pharmaceutically acceptable salt or in-vivo hydrolysable ester thereof:



(XVII)

Further suitable β-lactamase inhibitors include 6β-bromopenicillanic acid and salts and in-vivo hydrolysable esters and β-iodopenicillanic acid and salts and in-vivo hydrolysable esters thereof.

Such compositions of this invention comprising a β-lactamase inhibitor are formulated in conventional manner.

The present invention also includes a method of treating bacterial infections in humans and animals which comprises the administration of a therapeutically effective amount, more particularly an antibacterially effective amount, of an antibiotic compound of this invention.

The antibiotic compounds of the present invention are active against a broad range of gram-positive and gram-negative bacteria, in particular they are useful for treatment of respiratory tract and urinary tract infections in humans and mastitis in cattle.

The antibiotic compounds of the present invention are active against a wide range of gram-negative and gram-positive organisms including E.coli such as, for example ESS, JT4, JT425 and NCTC 10418; Pseudomonas Spp., such as Ps. aeruginosa, for example 100662 and Dalgleish; Serratia marcescens US32; Klebsiella aerogenes A; Enterobacter cloacae N1; P.mirabilis, such as, for example C977 and 889; P.morganii; P.rettgeri; B.subtilis; Staph. aureus such as, for example Oxford and Russell; N.catarrhalis 1502; Strepfaecalis I; and Strep. pyogenes CN10. The MIC data included in the following examples is representative of the activity of the compounds of the present invention.

The following examples illustrate the preparation and use of the compounds of the present invention.

Example 1

6β-[D,2-(2-p-Acetylaminophenyl-3-pyrazolin-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

(a) 2-p-Aminophenyl-3-pyrazolin-5-one-4-carboxylic acid.

2-p-Aminophenyl-4-ethoxycarbonyl-3-pyrazolin-5-one (1.45 g., 5.8mmol), prepared as described in UK Patent Application 8209426, in 0.5N sodium hydroxide solution (29 ml) was heated on a boiling water bath for 90 mins. The solution was then cooled, acidified to pH 3.5 (5N HCl) and the precipitate filtered, water washed and dried to give the title product (1.25 g, 97%), mp 207-8°, ν_{max} (nujol) 3370, 1665, 1580cm^{-1}, $\delta((CD_3)_2SO)$ 6.79 (2H,d, J 8Hz, aryl protons, o-to amino), 7.54 (2H,d,J 8Hz, aryl protons, m-to amino), 8.16 (1H,s, pyrazole proton).

(b) 2-p-Acetylaminophenyl-3-pyrazolin-5-one-4-carboxylic acid.

The acid (75 mg, 0.34 mml), prepared as described in (a) above, was heated at reflux in hexamethyldisilazane (1 ml) for 30 mins., and the solution evacuated. The solid residue was dissolved in dichloromethane (3 ml) and treated with acetyl chloride (0.025 ml, 0.35mmol) in dichloromethane (0.5 ml). The mixture was stirred 2 h. at room temperature; dilute HCl was then added to give a pH of 1 (aqueous phase) and the mixture stirred 15 min. Sodium bicarbonate solution and ethyl acetate were then added and the aqueous layer (pH 9) shaken, separated, acidified to pH 1.5 (5N HCl) and extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave the title product (60 mg, 67%), ν_{max} (nujol) 3270, 2400-3200, 1660 cm^{-1}, $\delta((CD_3)_2SO)$ 2.14 (3H,s,

-N-$COCH_3$), 7.75 (4H,s, aryl protons), 8.66 (1H,s,pyrazole proton), 10.11 (1H,s,exch. D_2O, -NH-). Found: M^+, 261.0741; $C_{12}H_{11}N_3O_4$ requires M, 261,0748.

(c) 6β-[D,2-(2-p-Acetylaminophenyl-3-pyrazolin-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt

The pyrazole acid, prepared as in (b) above (104 mg, 0.4 mmol) was suspended in dichloromethane (5 ml) and treated with triethylamine (0.08 ml, 0.57 mmol). The solution was cooled to -20° and treated with thionyl chloride (0.033 ml, 0.46 mmol) in dichloromethane (0.5 ml) and the mixture stirred 30 mins. at -10°. This solution was then added to a preformed solution of ampicillin (0.13 g, 0.37 mmol) with triethylamine (0.12 ml, 0.86 mmol) in dichloromethane (5 ml) at 0°. After 2 hours at room temperature, the mixture was treated with 1 drop 5N HCl and the volume concentrated. Water and ethyl acetate were added and the pH of the aqueous layer raised to 7.5 with dilute sodium bicarbonate solution. This layer was shaken, separated, acidified to pH 1.5 (5N HCl) and extracted with ethyl acetate to give the crude product(0.11 g). The title penicillin was purified as the free acid by chromatography on silica gel (ethyl acetate (5):isopropyl alcohol (4): water (1)), δ (CD_3OD) 1.47, 1.56 (6H, 2s, $(CH_3)_2$), 2.13 (3H, s, CH_3CO), 4.33 (1H, s, C_3-penicillanic proton), 5.44 (1H, d, J 4Hz, C_5-penicillanic proton), 5.57 (1H, d, J 4Hz, C_6-penicillanic proton), 5.78 (1H, s, -CHCON), 7.39 (5H, complex, ampicillin aryl protons), 7.63 (4H, s, pyrazole aryl protons), 8.36 (1H, s, pyrazole proton).

The free acid was dissolved in water by raising the pH to 6.9 with dilute sodium bicarbonate solution. Freeze-drying gave the title sodium salt (25 mg).
MIC against E. Coli NCTC 10418, 2.5 µg/ml

Example 2

6β-[D,2-(2-p-Acetylaminophenyl-3-pyrazolin-5-one-4-carbonylamino)-2-(4-hydroxyphenyl)]acetamido penicillanic acid, sodium salt

2-p-Acetylaminophenyl-3-pyrazolin-5-one-4- carboxylic acid, prepared as described in example 1(b), was converted to the title compound by the method of example 1(c), except that a preformed solution of amoxycillin triethylamine salt in dichloromethane was used instead of ampicillin. The amoxycillin triethylamine salt solution was prepared as follows:

Amoxycillin trihydrate (0.32 g, 0.76 mmol) in methanol (10 ml) was stirred with triethylamine (0.21 ml, 1.5 mmol) till dissolution occurred. Evaporation gave a white solid which was subsequently dissolved in dichloromethane.

The title product, first isolated as the free acid, possessed δ(CD_3OD) 1.48, 1.56 (6H, 2s, $(CH_3)_2$), 2.12 (3H, s, $-COCH_3$), 4.32 (1H, s, C_3-penicillanic proton), 5.42 (1H, d, J 4Hz, C_5-penicillanic proton), 5.55 (1H, d, J 4Hz, C_6-penicillanic proton), 5.65 (1H, s, -CHCON), 6.76 (2H, d, J 9Hz, aryl o-OH protons), 7.31 (2H, d, J 9Hz, aryl m-OH protons), 7.61 (4H, s, other aryl protons), 8.32 (1H, s, pyrazole proton). The free acid was dissolved in water by adding dilute sodium bicarbonate solution to pH 6.3. Freeze-drying gave the sodium salt.
MIC against E. Coli NCTC 10418, 2.5µg/ml

Example 3

6β-[D,2-([1,H]-1-[4-n-Butyramidophenyl -3-methoxy pyrazole-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

a) [1,H]-1-[4-Aminophenyl]-4-ethoxycarbonyl-3-methoxy Pyrazole

[1,H]-4-Ethoxycarbonyl-3-methoxy-1-[4-nitrophenyl] pyrazole (922mg, 3.17mmol) and 10% Palladium on charcoal catalyst (200mg) in dry dimethyl formamide (70ml) was hydrogenated at S.t.p for 2 hour. The catalyst was removed by filtration through celite and the filtrate evaporated to give the title compound after ether trituration (713mg) $\nu_{max}(CHCl_3)$ 1705, 1565, 1520, 1415, 1110cm^{-1}, δ (D_6-Acetone) 1.26(3H,t,J7Hz,-$OCH_2\underline{CH}_3$), 3.49(3H,s,-O$\underline{Me}$), 4.17 (2H,q, J7Hz, -$O\underline{CH}_2CH_3$), 4.78 (2H, brs,-$\underline{NH}_2$ [exchangeable D_2O]), 6.71 (2H,d, J9Hz, protons $\underline{o}$ to -NH_2), 7.51 (2H,d, J9Hz, protons $\underline{m}$ to -NH_2), 8.22 (1H,s,C_3-pyrazole proton). Found: M^+, 261.1102; $C_{13}H_{15}N_3O_3$ requires M, 261.1113.

b) [1,H]-1-[4-Aminophenyl]-3-methoxy pyrazole-4-carboxylic acid

[1,H]-1-[4-Aminophenyl]-4-ethoxycarbonyl-3-methoxy pyrazole prepared as in (a) above (900mg, 3.44mmol) was dissolved in methanol (30ml) and then treated with 0.5N sodium hydroxide solution (17ml) followed by stirring on a boiling water bath under nitrogen for 1.5 hours. The hot solution was then acidified to pH 4 (5NHCl) and allowed to cool. The methanol was removed by evaporation and the precipitate formed filtered and

washed with water, followed by drying under vacuum to give the title product (656mg); ν_{max} (Nujol) 1680, 1630, 1560, 1515, 1220, 1135cm^{-1}, δ (D_6-DMSO + CD_3OD) 3.92(3H, s, -OMe), 6.80 (2H,d,J.8Hz, protons o to $-NH_2$), 7.54 (2H,d,J8Hz, protons m to $-NH_2$), 8.48 (1H,s, C_3-pyrazole proton).

c) [1,H]-1-[4-n-Butyramido]phenyl-3-methoxy pyrazole -4-carboxylic acid.

The acid obtained in (b) above (520 mg, 2.23mmol) in hexamethyl disilazane (4ml) was refluxed under nitrogen for 30 mins. The excess hexamethyl disilazane was removed by evaporation and the residue evacuated overnight. The residue was dissolved in dry dichloro methane (20ml), cooled to 0°C under nitrogen and treated with butyryl chloride (260ul, 2.45mmol). The whole was allowed to warm to room temperature and stirred for 5 hours. The excess dichloromethane was subsequently evporated and water (25ml) at pH 2 admitted followed by stirring for 15 mins. The aqueous suspension was then basified to pH 9 with sodium bicarbonate solution and washed with ethylacetate. Reacidification of the aqueous layer to pH 1.5 (5.N HCl) produced a precipitate which was filtered, washed with water and dried under vacuum to give the title compound (519mg) ν_{max}(Nujol) 1710, 1660, 1610, 1580, 1215, 1130,cm^{-1}, δ(D_6-DMSO + CD_3OD) 1.00(3H,t,J6Hz, $-CH_2CH_2CH_3$), 1.70 (2H,m,$-CH_2CH_2CH_3$), 2.35(2H,m,$-CH_2CH_2CH_3$), 3.98(3H,s, -OMe), 7.75, (4H, Coincident singlet, aryl protons), 8.65 (1H,s, C_3-pyrazole proton).

d) 6β-[D,2-([1,H]-1-(4-n-Butyramido)phenyl-3-methoxy phenyl pyrazole-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

The title compound was prepared from the acid obtained in (c) above in a manner analogous to example 1(c). The crude product was purified as the free acid by column chromatography (SiO_2; 5:4:1 ethylacetate: isopropanol; water). The free acid possessed δ (D_6 Acetone +D_2O) 0.96 (3H,t,J6Hz, $-CH_2CH_2C\underline{H}_3$), 1.54(8H,m, $-CH_2C\underline{H}_2CH_3$, gemdimethyls), 2.39 (2H,m, $-C\underline{H}_2CH_2CH_3$), 4.13(3H,s, $-O\underline{C}H_3$), 4.34 (1H,s, C_3-penicillin proton), 5.64 (2H,m, C_5 and C_6-penicillin protons), 5.97 (1H,s,-C$\underline{H}$ CON-), 7.50 (5H,m,ampicillin phenyl protons), 7.82 (4H,s,pyrazole phenyl protons), 8.60(1H,s,C_3- pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 2. The sodium salt possessed ν_{max}(Nujol) 1765cm^{-1}.

MIC against E. Coli NCTC 10418, 16μg/ml.

Example 4

6β-[D,2-(2-(4-Benzamidophenyl)pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

a) 2-(4-Benzamidophenyl)-3-pyrazolin-5-one-4-carboxylic acid

The title compound was prepared from 2-(4-aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid in a manner analogous to example 1 (b), except that benzoyl chloride was used instead of acetyl chloride. The title compound possessed ν_{max} (Nujol) 1660, 1645, 1605, 1580, 1225, 1135, 825, 690cm, $^{-1}$ δ(D_6-DMSO + CD_3OD) 7.60 (9H,m, aryl protons), 8.45 (1H,s, C_3-Pyrazole proton).

b) 6β-[D,2-(2-(4-Benzamidophenyl)pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt

The title compound was prepared from 2-(4-benzamidophenyl)-3-pyrazolin-5-one-4-carboxylic acid obtained in (a) above in a manner analogous to example 5, except that ampicillin was used in place of amoxycillin. The free acid possessed δ(D_6-Acetone +D_2O), 1.44, 1.54 (2x3H, 2s, gem dimethyls), 4.28 (1H,s, C_3- penicillin proton), 5.40 (1H,d,J4Hz, C_5-penicillin proton), 5.60(1H,d,J 4Hz, C_6-penicillin proton), 5.95 (1H,s, -CHCON-), 7.60 (14H,m,aromatic protons), 8.50(1H,s, C_3-pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 2. The sodium salt possessed ν_{max} (Nujol) 1765cm^{-1}.
MIC against E. Coli NCTC 10418, 0.25μg/ml

Example 5

6β-[D,2-(2-(4-Benzamidophenyl) pyrazol-3-in-5-one-4-carbonylamino)-2-(4-hydroxyphenyl)]acetamido penicillanic acid, sodium salt

The title compound was prepared in an analogous manner to example 7, except that the carboxylic acid prepared in example 4(a) was used in coupling to amoxycillin. The crude product was purified as the free acid by column chromatography (SiO_2; 5:4:1 ethyl acetate : isopropanol : water). The free acid possessed δ (D_6-Acetone +D_2O) 1.48, 1.57 (2x3H, 2s, gem dimethyls), 4.38 (1H,s, C_3-penicillin proton), 5.60 (2H,m, C_5 and C_6-penicillin protons), 5.89 (1H,s, -CHCON-), 6.80 (2H,d,J9Hz, protons o-to-OH), 7.20-8.20(11H,m, aromatic protons), 8.55 (1H,s, C_3-pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 2. The sodium salt possessed ν_{max} (Nujol) 1760cm^{-1}.

MIC against E. Coli NCTC 10418, 0.25μg/ml

Example 6

6β-[D,2-(2-p-n-Butyramidophenyl pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

(a) 2-p-n-Butyramidophenylpyrazol-3-in-5-one-4-carboxylic acid.

2-p-Aminophenylpyrazol-3-in-5-one-4-carboxylic acid (0.22g, 1mmol) in hexamethyl disilazane (3ml) was refluxed for 30 mins. and the clear solution evacuated to dryness. The residue was dissolved in methylene chloride (5ml) and n-butyrl chloride (0.109ml, 1.05mmol) in methylene chloride (2ml) was added at 0°. After 2½ hours stirring at room temperature, water was added followed by 5N HCl(dropwise) till the pH of the stirred emulsion was 1.5. After 15 mins. stirring, sodium bicarbonate solution and ethyl acetate were added. The now basic aqueous layer was shaken, separated and acidified to pH 1.5. Ethyl acetate extraction, drying (Na_2SO_4) and evaporation gave the crude product which was triturated with ether to give (0.19g) product recrystallised from aqueous methanol, mp 248°(decomp). The product possessed ν_{max}(Nujol) 3300, 3150, 2400-3500(br), 1650cm^{-1}, δ (d-MeOH) 0.99(3H,t,J6Hz, CH_3-), 1.70 (2H, m, C-CH_2-C), 2.35(2H,t,J6.5Hz, CH_2CO-), 7.65 (4H,s,aryl protons), 8.39 (1H,s, pyrazole proton).

(b) Benzyl 6β-[D,2-(2-p-n-butyramidophenyl pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanate.

The carboxylic acid prepared in (a) above (125mg, 0.43mmol) in dry methylene chloride (5ml) with triethylamine (0.08ml, 0.57mmol) was cooled to -20° and treated with thionyl chloride (0.033ml, 0.46mmol) in methylene chloride (0.5ml). The solution was stirred 10 mins. at -10°C. Trimethyl silyl chloride (64ul, 0.5mmol) in methylene chloride (0.5ml) and triethylamine (0.07ml, 0.5mmol) were sequentially added and the solution stirred 5 min at -20°. The p-toluene sulphonic acid salt of ampicillin benzyl ester (0.35g, 0.57mmol) was converted to the free amine by partitioning the salt between ethyl acetate and sodium bicarbonate solution, and drying (Na_2SO_4) and evaporation of the organic layer. The residue (0.19g) was dissolved in MDC (5ml) at 0° and treated with triethylamine (0.06ml, 0.43mmol) and the above pyrazolinone solution. The mixture was stirred 2 hours at room temperature. Ethanol (1ml) was added, followed by a few drops of 5NHCl to lower the pH to 1.5. After 5 mins. stirring, excess ethyl acetate and water were added, the pH of the aqueous layer readjusted to 1.5 (HCl) and the organic layer shaken, separated, washed with brine, dried and evaporated to give the crude product (0.26g) which was purified by chromatography on silica (30g; ethylacetate (12): hexane (8): ethanol (1)). The title compound (65mg) possessed δ ($(CD_3)_2CO$) 0.97 (3H,t,J7Ht, CH_3-C), 1.36, 1.52(2x3H,2s, $(CH_3)_2C$), [obscuring 2H,m, Me-CH_2-C], 2.36(2H,t,J6Hz, -CH_2CO), 4.42 (1H,s,C_3-proton), 5.22(2H,s,-OCH_2), 5.52(1H,d,J4Hz, C_5-proton), 5.69(1H,d of d, J4, 8Hz, C_6-proton), 6.04 (1H,d,J7Hz, α-proton), 7.39 (10H, complex, aryl protons),

7.70 (4H, complex, aryl protons), 8.27 (2H,2d, J7, 8Hz, exch. D_2O, -NHCO), 9.26 (1H,s, exch. D_2O, Pr^nCONH-).

c) 6β-[D,2-(2-p-n-Butyramidophenyl pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

The penicillin ester prepared in (b) above (70mg) in tetrahydrofuran (2ml) was hydrogenated at S.T.P. over 10% palladium on charcoal (70mg) for 5½ hours. During the hydrogenation, fresh catalyst (70mg) was added (after 2 hour). The mixture was filtered through celite and the catalyst washed with tetrahydrofuran. Evaporation gave the title product as the free acid (40mg), δ ($(CD_3)_2CO$ + D_2O) 0.97 (3H,t, J7Hz, CH_3-C), 1.48 1.57 (6H,2s, $(CH_3)_2C$), partially obscured (2H,m, $MeCH_2$-), 2.40 (2H,t,J7Hz, CH_2CO), 4.31(1H,s,C_3- proton), 5.48(1H,s,J4Hz, C_5-proton), 5.62(1H,d,J4Hz, C_6-proton), 5.90(1H,s,α- proton), 7.38, 7.67(9H, complex, aryl protons), 8.46(1H,s, pyrazole proton).
The free acid was dissolved in water by shaking and adding sodium bicarbonate solution till the pH was 6.5 The aqueous solution was washed with ether and freeze dried to give the sodium salt, ν_{max} 1765cm^{-1}.

MIC against E. Coli NCTC 10418, 0.5μg/ml.

Example 7

6β-{D,2-(2-p-n-Butyramidophenyl pyrazol-3-in-5-one-4-carbonylamino)-2(4-hydroxyphenyl)}acetamido penicillanic acid sodium salt

2-p-n-Butyramidophenylpyrazol-3-in-5-one-4-carboxylic acid, prepared as described in example 6 (a), (145 mg, 0.5 mmol) in methylene chloride (5 ml) with triethylamine (0.07 ml, 0.5 mmol) was cooled to -20° and treated with thionyl chloride (0.04 ml, 0.56 mmol) in methylene chloride (0.5 ml). After 10 min at -10°, trimethylsilylchloride (0.13 ml, 1.0 mmol) in methylene chloride (0.5 ml) and triethylamine (0.16 ml, 1.14 mmol) was sequentially added, and the mixture stirred 5 min. at -20°.

Amoxycillin trihydrate (0.21 g, 0.5 mmol) in dry methanol (10 ml) was treated with triethylamine (0.14 ml, 1 mmol) and stirred till dissolution occurred. The solution was then evacuated. The resultant amoxycillin triethylamine salt was dissolved in methylene chloride (20 ml) with triethylamine (0.07 ml, 0.5 mmol). The solution was cooled to 0° and the above pyrazole solution added. The mixture was stirred 2 hours at room temperature, and the solvent volume reduced by evaporation. Ethyl acetate (excess) and water were added, and the pH raised to 7.5 with sodium bicarbonate solution. The aqueous layer was shaken, separated, acidified to pH 1.5 (5N HCl) and extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave the crude free acid of the title product (0.15 g) which was purified as follows. The material was chromatographed on silica gel (20 g) in ethyl acetate: isopropyl alcohol:water (5:4:1). The fractions containing the desired product was concentrated and water then added. The pH was raised to 7.5 with sodium bicarbonate solution

and the aqueous mixture washed with ethyl acetate and then acidified to pH 1.5 (HCl) and extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave essentially pure title product as the free acid, $\delta\{(CD_3)_2CO+D_2O\}$ 0.96 (3H, t, J 7Hz, CH_3-), 1.49, 1.58 (2 x 3H, 2s, $(CH_3)_2C$), 1.1-1.9 (2H, m, $MeCH_2$-), 2.39 (2H, t, J 7Hz, $-CH_2CO-$), 4.31 (1H, s, C_3-penicillin proton), 5.54 (1H, d, J 4Hz, C_5-penicillin proton), 5.70 (1H, d, J 4Hz, C_6-penicillin proton), 5.83 (1H, s, α-proton), 6.83 (2H, d, J8Hz, amoxycillin derived aryl protons, o-to-OH), 7.37 (2H, d, J 8Hz, other amoxycillin derived aryl protons), 7.71 (4H, s, other aryl protons), 8.50 (1H, s, pyrazole proton). The free acid was converted to the sodium salt by dissolution in water on shaking and adding dilute sodium bicarbonate solution till the pH=5.8, followed by freeze-drying.

MIC against E.Coli NCTC 10418, 0.5µg/ml.

Example 8

6β-[D,2-(2-[4-(3,4-Diacetoxybenzamido)phenyl]-3-pyrazolin-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

a) 2-(4-[3,4-Diacetoxybenzamido]phenyl)-3-pyrazolin-5-one-4-carboxylic acid

2-(4-Aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid (219 mg, 1 mmol) in hexamethyl disilazane (4 ml) was refluxed under nitrogen for 30 mins. The excess silylating agent was then removed by evaporation and the residue evacuated overnight.

3,4-Diacetoxybenzoic acid (238 mg, 1 mmol) in dry dichloromethane (5 ml) was treated with triethylamine (140 ml, 1 mmol) and stirred till dissolution occurred under nitrogen. The solution was then cooled to -30°C and treated with thionyl chloride (87 µl, 1.1 mmol) in dry dichloromethane (1 ml) followed by stirring for 1.5 hours.

The silylated amino acid from above was dissolved in dry dichloromethane (7 ml), cooled to -30°C and treated with the acid chloride solution; the whole was then allowed to warm to room temperature and stirred overnight. The excess dichloromethane was removed by evaporation and the residue dissolved in ethyl acetate and extracted with dilute sodium bicarbonate solution at pH 8.5. Acidification of the sodium bicarbonate solution to pH 1.5 (5N HCl) followed by ethyl acetate extraction, produced after drying ($MgSO_4$) and evaporation, the title compound (166 mg) ν_{max} (Nujol) 1770, 1680, 1650, 1580 cm^{-1}. δ(D_6-Acetone + D_6 DMSO + D_2O), 2.32 (6H, s, 2 x $\underline{CH_3}\overset{O}{\overset{\|}{C}}-O$), 7.30-8.20 (7H, m, aryl protons), 8.63 (1H, s, C_3-pyrazole proton).

b) 6β-{D,2-(2-{4-(3,4-Diacetoxybenzamido)phenyl}pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl}acetamido penicillanic acid, sodium salt

The acid prepared in (b) above (133 mg, 0.30 mmol) in dry dichloromethane (7 ml) was treated with triethylamine (48 µl, 0.34 mmol) and stirred in solution under nitrogen. The solution was cooled to -30°C and treated with thionyl chloride (26 µl, 0.33 mmol) in dry dichloromethane (1 ml). After stirring for 20 mins at -30°C a precipitate formed which was treated with chlorotrimethylsilane (39 µl, 0.30 mmol) in dry dichloromethane (1 ml) and triethylamine (43 µl, 0.30 mmol) to give a clear solution.

Simultaneously ampicillin (106 mg, 0.30 mmol) in dry dichloromethane (10 ml) was treated with triethylamine (86 µl, 0.60 mmol) and stirred at room temperature till dissolution occurred.

The ampicillin solution was cooled to -30°C and treated with the silylated acid chloride from above and the whole then allowed to warm to room temperature and stirred for 2 hours. The dichloromethane was removed by evaporation and the residue dissolved in ethyl acetate followed by extraction with dilute sodium bicarbonate solution at pH 7.7. The aqueous extract was then acidified to pH 1.5 (5N HCl) and extracted with ethyl acetate to give after drying ($MgSO_4$) and evaporation the crude title product as the free acid (117 mg). This was purified as the free acid by column chromatography (SiO_2, 5:4:1 ethyl acetate:isopropanol:water). The product possessed δ(D_6 Acetone + D_2O), 1.46, 1.56 (2 x 3H, 2s, gem dimethyls), 2.32 (6H, s, $\underline{CH}_3COO$-), 4.31 (1H, s, C_3-penicillin proton), 5.61 (2H, ABq, J 4Hz. C_5 and C_6-penicillin protons), 5.97 (1H, s, -C$\underline{H}$-CON-), 7.2-8.2 (12H, m, aryl

protons), 8.61 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 2. The sodium salt possessed ν_{max} (Nujol) 1765 cm^{-1}.

MIC against E.Coli NCTC 10418, 0.25µg/ml.

Example 9

6β-[D,2-(1,H-3-Acetoxy-1-[4-benzamido phenyl]-pyrazole -4-carbonyl amino)-2- (3,4-diacetoxyphenyl)] acetamido penicillanic acid sodium salt.

a) D,2-(2-[4-Benzamidophenyl] pyrazol-3-in-5-one-4-carbonylamino)-2-(3,4-dihydroxyphenyl) acetic acid.

D-3,4-Dihydroxyphenyl glycine (732mg, 4.0mmol) in hexamethyldisilazane (9ml) and chlorotrimethylsilane (3 ml) was stirred at reflux under nitrogen for 5 hours. The solution was allowed to cool and the solids filtered. The excess silylating agents were removed by evaporation and the oil evacuated overnight. 2-(4-Benzamidophenyl)-3-pyrazolin-5-one-4-carboxylic acid, obtained as in example 4(a)(646mg, 2.0mmol) in dry dichloromethane (20ml) was treated under nitrogen at room temperature with triethylamine (280ul, 2.0 mmol) and stirred to solution. The solution was cooled to -25°C and treated with thionyl chloride (162ul, 2.2mmol) in dry dichloromethane (1ml) and then stirred at -25°C for 20 mins to give a precipitate. Chlorotrimethylsilane (254ul, 2.0mmol) in dry dichloromethane (1ml) was then added followed by triethylamine (280ul, 2.0mmol) to give a clear solution. The per-silylated dihydroxyphenyl glycine obtained above was dissolved in dry dichloromethane (20ml), cooled to -25°C and treated with the silylated acid chloride from above. The whole was then allowed to warm to room temperature and stirred for 2 hours. The reaction solution was then concentrated by evaporation and water, adjusted to pH 2 by HCl(5N) addition, admitted followed by stirring for 20 mins at room temperature. The pH was

then adjusted to 8 with sodium bicarbonate solution and the aqueous solution washed with ethyl acetate followed by acidification to pH 1.5 (5NHCl). Ethyl acetate extraction of this produced, after drying ($MgSO_4$) and evaporation, the title compound, (443mg). ν_{max}(Nujol) 1720, 1640, 1595, 1515cm^{-1} δ(D_6-Acetone + D_2O) 5.46(1H,s,-CHCOOH), 6.84 (3H,m, 3 aryl protons), 7.20-8.15(9H,m,9aryl protons), 8.43(1H,s,C_3pyrazole proton).

b) D,2-(1,1-3-Acetoxy-1-[4-benzamidophenyl]-pyrazole-4-carbonylamino)-2-(3,4-diacetoxyphenyl) acetic acid.

The acid prepared as in (a) above (431mg, 0.88mmol) was dissolved in distilled tetrahydrofuran (3ml), and water (40ml) was added. Dilute bicarbonate solution was then added to give, at pH 7.8, a clear solution. The pH was then adjusted to 7.5 (HCl) and the solution cooled to 0°C and treated with acetic anhydride (420ul, 4.4mmol) in distilled tetrahydrofuran (2ml). Addition caused the pH to drop rapidly and it was maintained in the 7.1 - 7.5 region with dilute sodium bicarbonate solution. After 45 mins the pH remained static at 7.18. The solution was washed with ethyl acetate and then acidified to pH 1.5 (5N HCl). Ethyl acetate extration produced, after drying ($MgSO_4$) and evaporation, the title product (377mg). ν_{max}($CHCl_3$) 1770, 1710, 1660, 1605, 1565, 1610, 1180cm^{-1}. δ (D_6-Acetone +D_2O) 2.25 (6H,s, aryl acetoxy -CH_3), 2.36 (3H,s, pyrazole acetoxy -CH_3), 5.67(1H,s, -CHOOH), 7.0 - 8.2 (12H,m, 12aryl protons), 8.78 (1H,s,C_3pyrazole proton).

c) Benzyl 6β-[D,2-(1,H-3-Acetoxy-1-[4-benzamido phenyl] pyrazole-4-carbonylamino)-2-(3,4-diacetoxy phenyl)] acetamido penicillanate.

The acid prepared as in (b) above (308mg, 0.48mmol) in dry tetrahydrofuran (6ml) was treated with N-methylmorpholine (53ul, 0.48mmol) and the solution then cooled to -20°C under nitrogen and treated with methylchloroformate (38ul, 0.48mmol) in dry tetrahydrofuran (1 ml). This was stirred for 40 mins at -20°C and then added dropwise to a solution of benzyl 6β-amino penicillanate (148mg, 0.48mmol) in dry tetrahydrofuran (10 ml) at -20°C. The whole was then allowed to warm to room temperature and stirred for 2 hours. The reaction mixture was diluted with ethyl acetate, washed with water at pH 1.5, and then with brine. The organic layer was dried ($MgSO_4$) and evaporated to yield a crude product (430mg). This was purified by chromatography (SiO_2, 2:1 dichloromethane : Ethylacetate) to give the title compound (115mg), δ (D_6-Acetone) 1.34, 1.46 (2x3H, 2s, gemdimethyl), 2.22 (6H,s, aryl-$OCOCH_3$), 2.35 (3H,s,-$OCOCH_3$), 4.41(1H,s,penicillin C_3 proton), 5.20 (2H,s, $COOCH_2Ph$), 5.60 (2H,m, C_5 and C_6-penicillin protons), 5.88 (1H,d, J7Hz, -CH CON-), 7.10-8.20 (17H, m, aryl protons), 8.30 (1H,d, J7Hz, -NH), 8.84 (1H,s,C_3 pyrazole proton), 9.65 (1H, bs, -NH).

d) 6β-[D,2-(1,H-3-Acetoxy-1-[4-benzamido phenyl] pyrazole-4-carbonylamino)-2-(3,4-diacetoxy phenyl)] acetamido penicillanic acid, sodium salt.

The benzyl ester prepared in (c) above (115mg 0.12mmol) in tetrahydrofuran (10ml) was treated with 10% palladium/charcoal catalyst (115mg) and hydrogenated at S.T.P. for 45 mins. A further portion of 10% palladium/charcoal catalyst (115mg) was then added

and the whole hydrogenated at S.T.P. for a further 2.75 hours. The catalyst was then removed by filtration and the filtrate evaporated to dryness to yield the title product (42mg). The free acid possessed δ (D_6-Acetone + D_2O) 1.49, 1.52 (2x3H, 2s, gem dimethyl), 2.27 (6H,s, aryl-$OCOCH_3$), 2.39 (3H,s, -$OCOCH_3$) 4.34(1H,s, C_3-penicillin proton), 5.58 (2H,m, C_5 and C_6-penicillin protons), 6.00 (1H,s, -CHCON-), 7.10-8.40 (12H,m, aryl protons), 8.99 (1H,s, C_3- pyrazole proton). The free acid was converted to the sodium salt by suspension in water and addition of dilute sodium bicarbonate solution until, on shaking, dissolution occurred at pH 6.4, and freeze-drying (35mg).

MIC against E.Coli NCTC 10418, $\leq$ 0.06µg/ml.

Example 10

6β-[D,2-(2-[4-Benzamidophenyl]pyrazol-3-in-5-one-4-carbonylamino)-2-(3,4-diacetoxyphenyl)] acetamido penicillanic acid sodium salt.

6β-[D,2-(1,H-3-Acetoxy-1-[4-benzamido phenyl] pyrazole -4-carbonyl amino)-2-(3,4-diacetoxyphenyl)]acetamido penicillanic acid, sodium salt (35mg, 0.04mmol) was dissolved in water (4ml), cooled to 0°C and treated with n-butylamine (4ul, 0.04mmol), followed by stirring for 25 mins. at 0°C. The pH was then raised to 7.5 with dilute sodium bicarbonate solution and the aqueous solution washed with ethylacetate followed by acidification to pH 1.5 (5N HCl). Ethyl acetate extraction produced, after drying ($MgSO_4$) and evaporation 25mg of the title product. The free acid possessed δ (D_6-Acetone + D_2O) 1.50, 1.57 (2 X 3H, 2s, gem dimethyls), 2.30 (6H,s, CH_3COO-), 4.37 (1H,s,C_3 penicillin proton), 5.62 (2H,m, C_5 and C_6 penicillin protons), 6.04(1H,s, -CHCON-), 7.20-8.30 (12H, m, aryl protons), 8.66 (1H,s,C_3 pyrazole proton). The free acid was converted to the sodium salt by suspension in water and addition of dilute sodium bicarbonate solution, until on shaking at pH 6.5 dissolution occurred, and then freeze drying.

MIC against E.Coli NCTC 10418, 0.06μg/ml

Example 11

6β-[D,2-([1,H]-3-Acetoxy-1-[4-n-butyramidophenyl] pyrazole -4-carbonylamino)-2-(3,4-diacetoxyphenyl)] acetamido penicillanic acid, sodium salt

(a) D,2-(2-[4-n-Butyramidophenyl] pyrazol-3-in-5-one-4-carbonylamino)-2-(3,4-dihydroxyphenyl) acetic acid

D-3,4-Dihydroxyphenyl glycine (0.33 g, 1.8 mmol) in hexamethyl disilazane (9 ml) : trimethylsilylchloride (2.25 ml) was stirred at reflux under nitrogen for 3½ hours. The cooled solution was filtered from solid residues and evacuated overnight.

2-(4-n-Butyramidophenyl)-3-pyrazolin-5-one-4-carboxylic acid, obtained in Example 6(a) (0.22 g, 0.76 mmol) in dry methylene chloride (10 ml) with triethylamine (0.115 ml, 0.77 mmol) was cooled to -20°C and treated with thionyl chloride (0.056 ml, 0.75 mmol) in methylene chloride (2 ml). After 10 mins at -10°C, trimethylsilyl chloride (192 ml, 1.49 mmol) in methylene chloride (2 ml) and triethylamine (0.20 ml, 1.44 mmol) were seqentially added, and the solution stirred 5 min at -20°C. The solution was then added to the above silylated amino acid, predissolved in methylene chloride (30 ml) at 0°, and the mixture stirred 2 hours at room temperature. The volume of solvent was reduced to ca. 10 ml by evaporation and water (20 ml) was added. The mixture was shaken for 15 min at pH 2 (obtained initially by dropwise addition of 5N HCl). Ethyl acetate and sodium bicarbonate solution were then added. The mixture was shaken and the aqueous layer separated and acidified (HCl) to pH 1.5. Ethyl acetate extraction, drying (Na_2SO_4) and evaporation gave the title product (0.35 g), δ($(CD_3)_2CO$) 0.95 (3H, t, J 7Hz, CH_3-), 1.69 (2H, m, $MeCH_2$-), 2.36

(2H, t, J 6.5 Hz, $-CH_2CO-$), 5.53 (1H, d, J 7.5 Hz, $-CH-CO_2$), 6.79 (3H, m, dihydroxyaryl protons), 7.57 (4H, s, other aryl protons), 8.45 (1H, s, pyrazole proton), 8.10 (1H, d, J 7.5 Hz, exch. D_2O, -NHCO-), 9.18 (1H, s, exch, D_2O, -NHCO-).

(b) D,2-([1,H]-3-Acetoxy-1-[4-n-butyramidophenyl] pyrazole -4-carbonylamino)-2-(3,4-diacetoxyphenyl) acetic acid

This compound was prepared in a manner analogous to that described in Example 9(b). The product possessed ν_{max} (CH_2Cl_2) 1770, 1710, 1660 cm^{-1}, $\delta((CD_3)_2CO)$ 0.93 (3H, t, J 7Hz, CH_3-C-), 1.71 (2H, m, $MeCH_2-$), 2.29 (2H, obscured, $-CH_2CO-$), 2.23 (6H, s, CH_3CO_2-aryl), 2.31 (3H, s, CH_3CO_2-pyrazole), 5.60 (1H, d, J 7 Hz, $-CH-CO_2$), 7.24(3H, m, diacetoxyaryl protons), 7.58 (4H, m, other aryl protons), 8.64 (1H, s, pyrazole proton), 7.70 (1H, d, J 7 Hz, exch. D_2O, -NH-), 9.11 (1H, s, exch. D_2O, -NH-).

(c) Benzyl 6β-[D,2-([1,H]-3-Acetoxy-1-[4-n-butyramidophenyl] pyrazole-4-carbonylamino)-2-(3,4-diacetoxyphenyl]acetamido penicillanate

The crude title product was obtained in a manner analogous to that described in example 9 (c).

Purification was effected by chromatography on silica gel (ethyl acetate elution) to give the title product, ν_{max} (CH_2Cl_2) 3400, 3300, 1780, 1750s, 1705s, 1690, 1640, 1565, 1505 cm^{-1}, $\delta((CD_3)_2CO)$ 0.93 (3H, t, J 7Hz, CH_3-C-), 1.33, 1.46 (8H, 2s, and m, $(CH_3)_2$ and $MeCH_2-$), 2.20, 2.32 (11H, 2s, and obscured, CH_3CO_2- and $-CH_2CON-$), 4.34 (1H, s, C_3-penicillin proton, 5.13 (2H, s, $-OCH_2-$), 5.48 (2H, m, C_5 and C_6- penicillin protons, 5.86 (1H, d, J 7Hz, -CHCON), 7.27, 7.61 (13H, 2s, and m, aryl protons and -NH-), 8.18 (1H, d, J 7Hz, -NH-), 8.67 (1H, s, pyrazole proton), 9.08 (1H, s, -NH-).

(d) 6β-[D,2-([1H],-3-Acetoxy-1-[4-n-butyramidopheny1] pyrazole-4-carbony1amino)-2-(3,4-diacetoxypheny1)] acetamido penicillanic acid, sodium salt

The title compound was prepared as the free acid in a manner analogous to that described in Example 9 (d). The free acid possessed δ($(CD_3)_2CO$) 0.96 (3H, d, J 7Hz, CH_3-C-), 1.50, 1.52 (2 x 3H, 2s, $(CH_3)_2$), 1.64 (2H, m, Me CH_2-), 2.23, 2.33 (9H, 2s, CH_3CO_2-) (obscuring 2H, $-CH_2CON-$), 4.28 (1H, s, C_3-penicillin proton), 5.47 (m, C_5-penicillin proton with upper doublet of C_6-penicillin proton), 5.60 (lower doublet, J 4Hz, of C_6-penicillin proton, d of d), 5.86 (1H, d, J 7Hz, -CHCO-), 7.30, 7.64 (8H, m, aryl and -NH- protons), 8.21 (1H, d, J 7Hz, -NH-), 8.70 (1H, s, pyrazole proton), 9.14 (1H, s, -NH-). The free acid was converted to the sodium salt in the usual way.

MIC against E.Coli NCTC 10418, $\leq$ 0.06μg/ml

Example 12.

6β-[D,2-(2-(4-n-butyramidophenyl)pyrazol-3-in-5-one-4-carbonylamino)-2-(3,4-diacetoxyphenyl)] acetamido penicillanic acid

The penicillin prepared in Example 11 (d) (14 mg, 0.018 mmol) in water (2 ml) was treated with n-butylamine (0.002 ml) at room temperature. After 45 min, the pH was raised to 7.5 with dilute sodium bicarbonate solution and the aqueous solution washed with ethyl acetate, separated and acidified (5N HCl) to pH2. The solution was extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave the desired product (10 mg), 0.99 (3H, t, J 7.5 Hz, CH_3-C-), 1.54, 1.58 (8H, 2s and obscured m, $(CH_3)_2$ and $MeCH_2$-, 2.28 (6H, s, $-OCOCH_3$), 2.38 (2H, t J 8Hz, $-CH_2CON$), 4.35 (1H, s, C_3- penicillin proton), 5.58 (2H, m, C_5 and C_6- penicillin protons, 6.03 (1H, d, J 7.5Hz, -CH-CON-), 7.44 (3H, m, diacetoxyaryl protons), 7.74 (4H, s, other aryl protons), 8.24 (1H, d, J 7.5 Hz, -NH-), 8.61 (1H, s, pyrazole proton), 9.24 (1H, s, -NH-). (-NH-protons exch. D_2O).

MIC against E.Coli NCTC 10418, 0.03µg/ml.

Example 13

6,β-[D,2-[2-(4-Benzoylaminophenyl)-3-pyrazolin -5-one-4-carbonylamino]-2-phenyl]acetamido-6, α-formamido penicillanic acid, sodium salt

2-(4-benzoylaminophenyl)-3-pyrazolin-5-one-4-carboxylic acid (0.5 mmol, 163 mg) prepared in example 4(a) above was dissolved in methylene chloride (10 ml) with triethylamine (0.55 mmol, 80 μl) and cooled to -20°C. Thionyl chloride (0.5 mmol, 40 μl) was added dropwise and stirring continued for 20 minutes, allowing the temperature to warm to -10°C. Trimethylsilylchloride (0.55 mmol, 75 μl) was added dropwise and then, after a further 5 minutes, triethylamine (0.56 mmol, 80 μl) was added. The solution was cooled to -20°C and run into the penicillin solution, prepared as follows:

6α-Formamido ampicillin (0.5 mmol, 196 mg) was suspended in methylene chloride (15 ml), treated with triethylamine (2mmol, 280 μl) for 30 minutes and then stirred with 4Å moleuclar sieves for 30 minutes. The filtered solution was cooled to -20°C and treated with the acid chloride solution prepared above. The reaction mixture was stirred for 2 hours, allowing to warm to room temperature, and then the methylene chloride was evaporated off. The residue was dissolved in ethyl acetate and washed with dilute hydrochloric acid solution (pH 1.5) before being extracted into dilute sodium bicarbonate solution (pH 7.5; 2 x 25 ml). The aqueous fractions were combined and washed with ethyl acetate and then acidified with dilute hydrochloric acid to pH 1.5. The crude product was extracted into ethyl acetate which was then dried and evaporated and purified by column chromatography (SiO_2, eluting with ethylacetate : propan-2-ol : water = 5 : 4 : 2). The desired solvent fractions were evaporated and the residue dissolved in dilute sodium bicarbonate solution (pH 7.5)

and washed with ethylacetate. The aqueous solution was acidified with hydrochloric acid to pH 1.5 and the product extracted into ethylacetate. The solvent was dried and evaporated and then the product was converted to its sodium salt by dissolving in water with dilute sodium bicarbonate solution to pH 6.7 followed by freezedrying.

The product possessed:-

ν_{max} (nujol) 1770 (β lactam)

$\Delta[D_2O]$ 0.95 + 1.37 [6H, 2s, gem dimethyls], 4.17 [1H, s, C-3 proton], 5.59 + 5.57 [2H, 2s, C-α and C-5 proton], 7.53 [14H, complex, aromatic protons], 8.10 + 8.13 [2H, 2s, pyrazole proton and formamido proton].

MIC against E.Coli NCTC 10418, 1.0μg/ml.

Example 14. 6β-[D,2-([1,H]-3-Acetoxy-1-[4-benzoylaminophenyl]-pyrazole-4-carbonylamino)-2-(4-hydroxyphenyl)]acetamido-6α-formamido penicillanic acid sodium salt

a) [1,H]-3-Acetoxy-1-(4-benzoylaminophenyl)-4-methylcarbonyloxy-carbonylpyrazole.

2-[4-Benzoylaminophenyl]-3-pyrazolin-5-one-4-carboxylic acid (650 mg, 2mmol) was dissolved in methylenechloride (20ml) with triethylamine (4mmol, 560µl) and cooled to 0°C. Acetylchloride (280µl, 4mmol) was run in and a pale precipitate appeared. The mixture was stirred for two hours, evaporated to dryness and then partitioned between dilute sodiumbicarbonate and ethylacetate. The organic phase was shaken, separated, dried ($MgSO_4$) and evaporated to give the title product, used in (b) without further purification. ν_{max} (CH_2Cl_2) 3420, 1810, 1800, 1775 cm^{-1}, δ(d-DMSO+D_2O) 2.40, 2.46 (6H, 2s, acetoxy and anhydride CH_3), 7.4-8.0 (9H, complex, aromatic protons), 9.03 (1H, s, pyrazole-5-proton).

b) [1,H]-3-Acetoxy-1-(4-benzoylaminophenyl)pyrazole-4-carboxylic acid.

The anhydride prepared in a) above was dissolved in 50% aqueous tetrahydrofuran (30ml) and treated with dilute sodium bicarbonate and dilute sodium hydroxide solutions to give pH 9.0; at this point the pH started to drop slowly and so the pH was maintained at 9.0 until hydrolysis stopped. After 2 hours, the aqueous layer was extracted with ethylacetate at pH 1.5. Drying ($MgSO_4$) and evaporation gave the title product (0.54g, 74% for steps (a) and (b), mp 227-230°.
ν_{max} (Nujol) 3340, 1780, 1700, 1660, 1530 cm^{-1}. δ[d^6-DMSO + d^6-acetone] 2.35 [3H, s, CH_3COO], 7.4-8.2(9H, complex, aromatic protons), 8.85 [3H, s, pyrazole C-5 proton].

c) Benzyl-6β[D,3-([1,H]-3-Acetoxy-1-[4-benzoylaminophenyl] pyrazole-4-carbonylamino)-2-(4-benzyloxycarbonyloxyphenyl)]acetamido-6α-formamido penicillanate.

Benzyl-6β[D,2-(4-benzyloxycarbonyloxyphenyl)-2-(2,2,2-trichloroethoxycarbonylamino)]acetamido-6α-formamido penicillanate (245mg, 0.3mmol) was dissolved in tetrahydrofuran (20ml) with potassiumdihydrogenphosphate solution (M. 4ml) at pH 4 and treated with fresh acid-washed zinc in several batches until the starting material was consumed (t.l.c). The resulting suspension was filtered and washed with tetrahydrofuran. The filtrate was evaporated to low volume and partitioned between brine and ethylacetate. The ethylacetate was dried ($MgSO_4$) and evaporated to give crude benzyl-6β-[D,2-amino-2-(4-benzyloxycarbonyloxyphenyl)]acetamido-6α-formamido penicillanate (0.16g), used below without further purification. [1,H]-3-Acetoxy-1-(4 benzoylaminophenyl)-pyrazole-4-carboxylic acid (0.25mmol, 92mg) was dissolved in methylene chloride (5ml) with triethylamine (35µl, 0.25mmol) and cooled to 10°C . Thionyl chloride (20µl, 0.25mmol) was added dropwise and the solution stirred for 5 mins. This was then added at -10° to a solution of the above α-amino penicillin ester in methylene chloride (20ml), with pyridine (20µl, 0.25mmol). After 45 mins, the solvent was concentrated and the residue dissolved in ethylacetate and washed with dilute hydrochloric acid, water, dilute sodiumbicarbonate and brine, and dried ($MgSO_4$) and evaporated. The title product was purified by eluting from a silica column with 10% hexane in ethylacetate. Yield 80mg (33%).

ν_{max} (CH_2Cl_2) 1785; 1760 cm^{-1}, δ(d^6-Acetone + D_2O) 0.97 + 1.20 [6H, 2s, gemdimethyls] 2.43 [3H, s, pyrazole acetoxy], 4.47 [1H, s, C-3 proton], 5.20, 5.27 [4H, 2s, benzyl CH_2], 5.70 (1H, s, C-5 proton), 5.90 (1H, s, -CHCON), 7.1-8.1 [18H, complex, aromatic protons], 8.23 [1H, s, NHCHO], 9.0 (1H, s, pyrazole C-5 proton].

d) 6β-[D,2-([1,H]-3-Acetoxy-1-[4-benzoylaminophenyl]pyrazole-4-carbonylamino)-2-(4-hydroxyphenyl)]acetamido-6,α-formamido penicillanic acid, sodium salt.

The product obtained in (c) above (80mg) was hydrogenated in tetrahydrofuran (10ml) with 10% palladium on charcoal (80mg) for one hour at S.T.P. when t.l.c. showed reaction to be complete. The catalyst was filtered off and the tetrahydrofuran evaporated. The residue was dissolved in dilute sodium bicarbonate solution, washed with ethylacetate, acidified to pH 1.5 and extracted into ethylacetate. The organic layer was dried ($MgSO_4$) and evaporated to give the title product as the free acid, converted to its sodium salt by dissolution in water by the addition of a dilute solution of sodium bicarbonate to pH 6.7, and freeze-drying (30mg).
ν_{max} (Nujol) 1765cm^{-1}. δ[d^6-Acetone + D_2O] 1.03, 1.33 [6H,2s, gemdimethyls], 2.40 [3H, s, $COCH_3$], 4.27 [1H, s, penicillin C-3 proton], 5.57 (1H, s, C-5 proton), 5.67 (1H, s, -CHCON), 6.7-8.0 [12H, complex, aromatic protons], 8. 02[1H, s, NCHO], 8.73 [1H, s, pyrazole C-5 proton].
M.I.C. against E. coli NCTC 10418, 4.0 μg/ml.

Example 15. 6β-[D,2-([1,H]-3-Acetoxy-1-(4-n-butyramidophenyl)-pyrazole-4-carbonylamino)-2-(3,4-diacetoxyphenyl)] acetamido bisnorpenicillanic acid, sodium salt.

a) Benzyl-6β-[D,2-([1,H]-3-acetoxy-1-[4-n-butyramidophenyl]-pyrazole-4-carbonylamino)-2-(3,4-diacetoxyphenyl)]acetamido bisnorpenicillanate.

The paratoluenesulphonic acid salt of benzyl 6β-aminobisnor penicillanate (100mg, 0.21mmol) was converted to its free base in dilute aqueous sodium bicarbonate and extracted into ethylacetate. After drying ($MgSO_4$) and evaporation of the organic phase the residue was dissolved in tetrahydrofuran (5ml) and cooled to -15°C.

D,2-([1,H]-3-Acetoxy-1-[4-n-butyramidophenyl]pyrazole-4-carbonylamino)-2-(3,4-diacetoxyphenyl)acetic acid (116mg. 0.2mmol) was dissolved in dry tetrahydrofuran (5ml) and treated with N-methyl morpholine (22µl, 0.2mmol). The solution was cooled to -15°C and methylchloroformate (16µl, 0.2mmol) added. After 15 mins this solution was added to the above solution of bisnor penicillanate at -15° and the whole stirred for 20mins at room temperature. The solvent was removed by evaporation and the residue partitioned between ethylacetate and water at pH 1.5 (dilute hydrochloric acid). The organic phase was washed quickly with water, dilute sodium bicarbonate solution, brine, dried ($MgSO_4$) and evaporated. The title product was purified by eluting from silica with 2:1 ethyl-acetate : hexane, to give 40mg (23%).

δ[d^6-Acetone] 0.95[3H, t, J7Hz, Butyryl CH_3],1.7[2H,m, butyryl $CH_2\underline{CH_2}CH_3$], 2.2 [butyryl $\underline{CH_2}CH_2CH_3$ under acetoxy],2.20 [6H, s, aromatic acetoxys], 2.33 [3H, s, pyrazole acetoxy], 3.40 [2H, d, J 5Hz, penicillin C-2 protons], 5.05 [1H, t, J 5Hz, penicillin C-3 proton], 5.13 [2H, s, benzyl CH_2], 5.30 [1H, d, J4Hz, penicillin C-5 proton], 5.53 [1H,d of d.J8, 4Hz, penicillin C-6 proton], 5.83 [1H, d, J7.5Hz, C-α proton],7.30 [5H, s, benzyl aromatic protons], 7.66 [4H, s, aromatic protons], 7.3-7.9 [3H, complex, aromatic protons], 8.2 (1H, d, J8Hz, penicillin C-6 NH], 8.70 [1H, s, pyrazole C-5 proton], 9.2[1H, s, NH-butyryl].

b) 6β-[D,2-([1-H]-3-Acetoxy-1-[4-*n*-butyramidophenyl]pyrazole-4-carbonylamino)-2-(3,4-diacetoxyphenyl)]acetamido bisnor penicillanic acid, sodium salt.

The benzyl ester prepared in (a) above (40mg) was hydrogenated in tetrahydrofuran (10ml) with 10% palladium on charcoal (80mg) for one hour at S.T.P. The catalyst was filtered off and washed with fresh tetrahydrofuran (10ml) and then the filtrate was evaporated. The residue was partitioned between ethyl acetate and dilute sodium bicarbonate solution at pH 7.5. The aqueous layer was acidified (pH 1.5; HCl) and extracted into ethylacetate. After drying ($MgSO_4$) and evaporation the title product was converted to its sodium salt by dissolving in water at pH 6.7 with dilute sodium-bicarbonate, and freeze dried (20mg).

δ[d^6-acetone] 0.93 [3H, t, butyryl CH_3], 1.70 [2H. m, butyryl $CH_2\underline{CH_2}CH_3$], 2.2 [butyryl $\underline{CH_2}CH_2CH_3$ under acetoxy groups]. 2.20 [6H, s, aromatic acetoxy],2.33 [3H, s, pyrazole acetoxy]. 3.36 [2H, d, J 4.5 Hz, penicillin C-2 protons]. 4.95 [1H. t, J 4.5 Hz., penicillin C-3 proton], 5.25 [1H, d, J 4Hz. penicillin C-5 proton], 5.57 [1H, d of d, J4 and 8Hz. penicillin C-6 proton]. 5.79 [1H, d, J 7.5 Hz, C-α proton],7.0-7.8 [7H, complex aromatic protons]. 8.15 [1H, d, J8 Hz. C-6 NH], 8.66 [1H, s, pyrazole C-5 proton], 9.1 [1H, broad, NH-butyryl].

M.I.C. against E. coli NCTC 10418, 0.12μg/ml

Example 16. 6β-[D,2-(2-p-Methylsulph nylaminophenyl pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

a) 2-p-Methylsulphonylaminophenyl pyrazole-3-in-5-one-4-carboxylic acid.

2-p-Aminophenylpyrazol -3-in-5-one-4-carboxylic acid (0.247g., 1.12mmol) in hexamethyl disilazane (10ml), was refluxed for 30 min. and the solution evacuated to dryness. The residue in methylene chloride (10ml) was treated with methane sulphonyl chloride (74µl, 0.92mmol) in methylene chloride (1ml), and the solution stirred at room temperature for 4 days. The solvent was evacuated and the residue dissolved in aqueous sodium bicarbonate solution. This was then acidified (5N HCl) to pH 1 and precipitated solids were removed by filtration. The remaining solution was extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave the title product (0.13g)., recrystallised from aqueous methanol, m.p.228°. (decarboxylation), ν_{max} (Nujol) 1660, 1340, 1150 cm^{-1}, λ_{max} (EtOH) 289nm, δ(d-DMSO) 2.99 (3H, s, $-CH_3$). 7.20 (2H, d, J 8.5 Hz, aryl protons). 7.68 (2H, d, J 8.5 Hz, aryl protons). 8.58 (1H, s, pyrazole proton), 9.74 (1H, s, exch. D_2O, -NH-). Found: M+, 297.0424. $C_{11}H_{11}N_3O_5S$ requires M, 297.0419.

b) 6β-[D,2-(2-p-Methylsulphonylaminophenyl pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

The pyrazole acid, prepared in (a) above, (40mg, 0.13mmol) in dry methylene chloride (5ml) was treated with trioctylamine (70µl, 0.16mmol) and the clear solution cooled to -20° and treated with $SOCl_2$ (10µl, 0.14mmol) in methylene chloride (0.5ml). After 20 min. at -10°, trimethylsilylchloride (17µl, 0.13mmol) in methylene chloride (0.5ml) and triethylamine (18µl, 0.13mmol) were added, and the mixture stirred 5 mins at -20°. The solution was then added to predissolved ampicillin (0.045g, 0.13mmol) in methylene chloride (2ml) with triethylamine (36µl, 0.26mmol). The addition was carried out at 0° and the mixture subsequently stirred for 2 hours at room temperature. Water and ethylacetate were added and the pH adjusted to 7.5. The aqueous layer was shaken, separated

acidified to pH 1.5 and extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave the title product as the free acid (40mg), δ(d-acetone + D_2O) 1.32, 1.41 (2 x 3H, 2s, $(CH_3)_2$), 3.01 (3H, s, CH_3SO_2-), 4.30 (1H, s, C_3-proton), 5.46 (1H, d, J4Hz, C_5-proton), 5.63 (1H, d, J 4Hz, C_6-proton), 5.75 (1H, s, -CHCON), 7.43 (9H, complex, aryl protons), 8.51 (1H, s, pyrazole proton). The free acid was converted to the sodium salt by dissolution in water by the addition of sodium bicarbonate solution to pH 6.5 and freeze-drying.M.I.C. against E. coli NCTC 10418, 1.0μg/ml.

Example 17

6β-[D,2-(2-p-Methoxycarbonylaminophenyl pyrazol -3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

(a) 2-p-Methoxycarbonylaminophenyl pyrazole-3-in-5-one-4-carboxylic acid.

2-p-Aminophenylpyrazole-3-in-5-one-4-carboxylic acid (0.12g. 0.55mmol) was stirred in methylene chloride (10ml) with triethylamine (0.23ml, 1.65mmol). After dissolution. trimethylsilyl chloride (0.215ml, 1.65mmol) in MDC (0.5ml) was added and the solution stirred 1 hour at room temperature. The solution was cooled to 0^{o} and methyl chloroformate (42µl, 0.55mmol) in methylene chloride (0.5ml) was added. After 3 hours stirring at room temperature, water was added and the pH adjusted to 2 with hydrochloric acid. The mixture was shaken 15 min and sodium bicarbonate solution added. The mixture was then diluted with excess ethyl acetate and the aqueous layer was separated, acidified to pH 1.5 and extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave the title product (0.09g). mp 244^{o}(decarbox.). ν_{max}(nujol) 1750, 1675 cm^{-1}. λ_{max}(EtOH) 290nm, δ(d-DMSO) 3.74 (3H. s. $-OCH_3$), 7.52 (2H. d, J10Hz. aryl protons). 7.72 (2H. d, J 10Hz. aryl protons). 8.62 (1H. s. pyrazole proton). 9.76 (1H. s, D_2O exch.. -NH-). Found: M^+. 277.0705. $C_2H_{11}N_3O_5$ requires M. 277.0699.

(b) 6β-[D,2-(2-p-Methoxycarbonylaminophenyl pyrazole-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid. sodium salt.

The pyrazole acid. prepared in (a) above (90mg. 0.32mmol) in dry methylene chloride (5ml) was treated with triethylamine (50µl. 0.35mmol), the solution cooled to -20^{o} and thionyl chloride (24µl. 0.32mmol) in methylene chloride (0.5ml) added. After 10mins. at -10^{o}, trimethylsilyl chloride (42µl, 0.32mmol) in methylene chloride (0.5ml) was added followed by triethylamine (45µl, 0.32mmol). The mixture was stirred 5 min. at -20^{o}. and then added to predissolved ampicillin (0.11g, 0.32mmol) in methylene chloride (5ml) with triethylamine (0.09ml, 0.64mmol) at 0^{o}. The reaction solution was stirred 2 hours at room temperature wh n water and excess ethyl

acetate were added and the pH raised to 7.5 with aqueous sodium bicarbonate solution. The aqueous layer was shaken, separated, acidified to pH 1.5 (HCl) and extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave the crude title product which was purified by chromatography on silica (ethyl acetate (5): isopropyl alcohol (4): water (1)). The desired free acid (50mg) possessed δ($(CD_3)_2CO$) 1.49, 1.56 (2 x 3H, 2s, $(CH_3)_2$), 3.67 (3H, s, $-OCH_3$), 4.29 (1H, s, C_3- penicillin proton), 5.45 (1H, d, J4Hz, C_5- proton), 5.63 (1H, d of d, J4, 8Hz, C_6- proton), 5.93 (1H, d, J8Hz, -CHCON), 7.56 (9H, complex, aryl protons), 8.13 (2H, 2d, J≈8Hz, -NH-, exch. D_2O),8.49 (1H, s, pyrazole proton), 8.66 (1H, s, -NH-, exch. D_2O). The free acid was converted to the sodium salt by dissolution in water by adding dilute sodium bicarbonate solution to give pH 6.2, shaking and freeze-drying. The sodium salt possessed ν_{max}(nujol) 1770, 1720, 1660, 1600 cm^{-1}.

M.I.C. against E. coli NCTC 10418, 1.0µg/ml.

Example 18: 6β-[D,2-(2-{4-N-Acetyl-D-alanylamino}phenyl pyrazol -3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

(a) 2-(4-N-Acetyl-D-alanylamino)phenylpyrazole-3-in-5-one-4-carboxylic acid.

To N-acetyl-D-alanine (65mg, 0.5mmol) in acetonitrile (2ml), N-methylmorpholine (0.055ml, 0.5mmol) was added. The solution was cooled to -15° and methyl chloroformate (40µl, 0.52mmol) added. After 30 mins. at -15°, this solution was added to the following mixture:-

2-p-Aminophenylpyrazol -3-in-5-one-4-carboxylic acid (0.12g., 0.55mmol) in methylene chloride (10ml) was treated with triethylamine (0.23ml, 1.65mmol) and trimethylsilyl chloride (0.215ml, 1.65mmol) in methylene chloride (0.5ml). The mixture was stirred 1 hour at room temperature, and then cooled to 0°. The above acetonitrile solution was added, and the mixture stirred 1 hour at room temperature. The solution was evaporated and the residue dissolved in aqueous bicarbonate solution. This was acidified to pH 1.2 to give the title product as a precipitate which was filtered and washed with water. The filtrate was then extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave further desired product (total weight, 0.10g), ν_{max} (nujol) 3280, 1690 (sh), 1670, 1650 cm^{-1}, δ(d-DMSO) 1.28 (3H, d, J 6.5 Hz, CH_3C), 1.84 (3H, s, CH_3CO-), 4.33 (1H, "quintet", "J" 6.5 Hz, -CHMe), 7.58 (4H, s, aryl protons), 8.05 (1H, d, J 6.5 Hz, -NH-C-Me), 8.49 (1H, s, pyrazole proton), 9.96 (1H, s, MeCONH-). mp 237° (decomp.). Found: M^+, 332.1122; $C_{15}H_{16}N_4O_5$ requires M, 332.1121.

(b) 6β-[D,2-(2-{4-N-Acetyl-D-alanylamino}phenyl pyrazole-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

The product prepared as in (a) above (0.30g., 0.88mmol) in methylene chloride (60ml) was treated with trioctylamine (0.42ml, 0.96mmol) and the solution cooled to -20° and treated with thionyl chloride (86µl, 0.96mmol) in methylene chloride (2ml). After 10 mins at -10°, trimethylsilyl chloride (0.115ml, 0.90mmol) in methylene chloride (2ml) and triethylamine (0.12ml, 0.88mmol) were sequentially added, and the mixture stirred 5 mins at -20°. The

solution was then added to ampicillin (0.304g., 0.88mmol), predissolved in methylene chloride (20ml) with triethylamine (0.24ml, 1.76mmol) at 0°. After 2 hours at room temperature, the mixture was concentrated by evaporation and water and ethyl acetate added. The pH was adjusted to 7.5 with dilute bicarbonate solution. The aqueous layer was shaken, separated, acidified to pH 1.5 and the precipitate filtered off and washed with water. Drying under vacuum gave the crude title product (0.22g) which was chromatographed on silica (40g.: ethyl acetate (5): isopropy l alcohol (4): water (2)) to give the desired penicillin (70mg) as the free acid, δ(CD_3OD) 1.40 (3H, t, J 7Hz, CH_3-C-N), 1.45, 1.53 (2 x 3H, 2s, $(CH_3)_2$), 1.97 (3H, s, CH_3CON), 4.27 (1H, s, C_3-proton), 4.42 (1H, q, J 7Hz, -CHNAc), 5.36 (1H, d, J 4Hz, C_5 -proton), 5.50 (1H, d, J4Hz, C_6-proton), 5.73 (1H, s, α-proton, 7.28 (5H, complex, aryl protons), 7.51 (4H, s, aryl protons), 8.27 (1H, s, pyrazole proton). The free acid was converted to the sodium salt in the usual way, ν_{max} (nujol) 1760, 1660, 1600 cm^{-1}.

M.I.C. against E. coli JT425, 4.0μg/ml.

Example 19: 6β-[D,2-(2-(4-Ethoxycarbonylaminophenyl) pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

(a) 2-(4-Ethoxycarbonylaminophenyl)-3-pyrazolin-5-one-4-carboxylic acid.

The title compound was prepared from 2-(4-aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid in a manner analogous to example 1 (b), except that ethyl chloroformate was used instead of acetyl chloride. The title compound possessed ν_{max}(Nujol) 1730, 1660, 1610, 1585, 1540, 1220 cm^{-1}, δ(D_6-Acetone + D_6-DMSO) 1.28 (3H, t, J6Hz, $-OCH_2\underline{CH_3}$), 4.13 (2H, q, J6Hz $-O\underline{CH_2}CH_3$), 7.57 (4H, s, aryl protons), 8.37 (1H, s, C_3 pyrazole proton).

(b) 6β-[D,2-(2-(4-Ethoxycarbonylamino phenyl) pyrazol-3-in-5-one-4-carbonyl amino)-2-phenyl] acetamido penicillanic acid, sodium salt.

The title compound was prepared from 2-(4-ethoxycarbonylamino-phenyl)-3-pyrazolin-5-one-4-carboxylic acid, obtained in (a) above, in a manner analogous to example17 (b). The free acid possessed δ(D_6-Acetone + D_2O) 1.23 (3H, t, J 7Hz, $-OCH_2\underline{CH_3}$), 1.45, 1.54 (2 x 3H, 2s, gemdimethyl), 4.16 (2H, q, J7Hz, $O\underline{CH_2}CH_3$), 4.34 (1H, s, C_3 penicillin proton), 5.60 (2H, ABq, J 4Hz, C_5 and C_6 penicillin protons), 6.00 (1H, s, -CHCON-), 7.50 (9H, m, aryl protons), 8.53 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17(b). The sodium salt possessed ν_{max} (Nujol) 1765 cm^{-1}.
M.I.C. against E. coli NCTC 10418, 1.0 μg/ml.

Example 20: 6β-[D,2-(2-[4-Phenylmethylcarbonylaminophenyl] pyrazol -3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

(a) 2-[4-Phenylmethylcarbonylaminophenyl]-3-pyrazolin-5-one-4-carboxylic acid.

The title compound was prepared from 2-(4-aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid in a manner analogous to example 1 (b) except that phenylacetylchloride was used instead of acetylchloride. The title compound possessed ν_{max} (Nujol) 1655, 1605, 1579, 1525, 1135 cm^{-1}. $\delta(D_6\text{-DMSO})$ 3.77 (2H, s, $PhCH_2CO$-), 7.41 (5H, s, $PhCH_2$-), 7.80 (4H, s, pyrazole aryl protons), 8.68 (1H, s, C_3 pyrazole proton). Found: M^+, 337.1061, $C_{18}H_{15}N_3O_4$ requires M, 337.1062.

(b) 6β-[D,2-(2-[4-Phenylmethylcarbonylaminophenyl]pyrazol -3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

The title compound was prepared from 2-(4-phenylmethylcarbonylamino phenyl)-3-pyrazolin-5-one-4-carboxylic acid, obtained in (a) above, in a manner analogous to example 17 (b). The free acid possessed $\delta(D_6\text{-Acetone} + D_2O)$ 1.46, 1.56 (2 x 3H, 2s, gemdimethyl), 3.69 (2H, s, $PhCH_2CO$-), 4.26 (1H, s, C_3 penicillin proton), 5.53 (2H, ABq, J4Hz, C_5 and C_6 penicillin protons), 5.89 (1H, s, -CHCON-), 7.05-7.80 (14H, m, aryl protons), 8.42 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17 (b). The sodium salt possessed ν_{max} (Nujol) 1765 cm^{-1}.

M.I.C. against E. coli NCTC 10418, 0.5μg/ml.

Example 21: 6β-[D,2-(2-[4-Methylaminocarbonylaminophenyl] pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

(a) 2-[4-Methylaminocarbonylaminophenyl]-3-pyrazolin-5-one-4-carboxylic acid.

The title compound was prepared from 2-(4-aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid in a manner analogous to example 1(b), except that methylisocyanate was used instead of acetyl chloride, and the reaction time was increased to 24h. The title compound possessed ν_{max} (Nujol) 1665, 1640, 1605, 1580, 1220 cm^{-1}, δ(d_6-Acetone + D_6-DMSO) 2.68 (3H, s, -NHC<u>H</u>$_3$), 5.95 (1H, bs, -N<u>H</u>, exchangeable D_2O), 7.50 (4H, coincidental singlet, 4 aryl protons), 8.44 (1H, s, C_3 pyrazole proton), 8.50 (1H, bs, -NH, exchangeable D_2O). Found: M^+, 276.0858; $C_{12}H_{12}N_4O_4$ requires M, 276.0858.

(b) 6β-[D,2-{2-(4-Methylaminocarbonylaminophenyl) pyrazol-3-in-5-one-4-carbonylamino}-2-phenyl]acetamido penicillanic acid, sodium salt.

The title compound was prepared from 2-(4-Methylaminocarbonyl-amino phenyl)-3-pyrazolin-5-one-4-carboxylic acid, in a manner analogous to example 17 (b). The free acid possessed δ(CD_3OD + D_6-Acetone) 1.47, 1.56 (2 x 3H, 2s, gemdimethyl), 2.76 (3H,s,-N<u>Me</u>), 5.60 (2H, ABq, J4Hz, C_5 and C_6 penicillin protons), 5.90 (1H, s, -C<u>H</u>CON-). 7.10-7.80 (9H, m, aryl protons), 8.41 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17 (b). The sodium salt possessed ν_{max} (Nujol) 1770 cm^{-1}.

M.I.C. against E. coli NCTC 10418, 1.0μg/ml.

Example 22: 6β-[D,2-Phenyl-2-(2-{4-n-proprionamidophenyl}pyrazol-3-in-5-one-4-carbonylamino)]acetamido penicillanic acid, sodium salt.

(a) 2-[4-n-Proprionamidophenyl]-3-pyrazolin-5-one-4-carboxylic acid.

The title compound was prepared from 2-(4-aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid in a manner analogous to example 1(b), except that proprionylchloride was used instead of acetyl chloride. The title compound possessed ν_{max} (Nujol) 3300, 1660, 1605, 1510 cm^{-1}, δ(D_6-DMSO + D_6-Acetone) 1.10 (3H, t, J7Hz, $-COCH_2\underline{CH_3}$), 2.35 (2H, q, J7Hz, $CO\underline{CH_2}CH_3$), 7.58 (4H, s, 4 aryl protons), 8.46 (1H, s, C_3-pyrazole proton). Found: M^+, 275.903; $C_{13}H_{13}N_3O_4$ requires M, 275.906.

(b) 6β-[D,2-Phenyl-2-(2-[4-n-proprionamidophenyl]-pyrazol-3-in-5-one-4-carbonylamino)] acetamido penicillanic acid, sodium salt.

The title compound was prepared from 2-(4-n-proprionamido-phenyl)-3-pyrazolin-5-one-4-carboxylic acid, obtained in (a) above, in a manner analogous to example 17 (b). The free acid possessed δ(D_6-Acetone + D_2O) 1.15 (3H, t, J8Hz, $COCH_2\underline{CH_3}$), 1.47, 1.56 (2 x 3H, 2s, gemdimethyl), 2.41 (2H, q, J8Hz, $CO\underline{CH_2}CH_3$), 4.27 (1H, s, C_3 penicillin proton), 5.51 (2H, ABq, J4Hz, C_5 and C_6 penicillin protons), 5.89 (1H, s, -CHCON-) 7.10-7.80 (9H, m, aryl protons), 8.38 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17 (b). The sodium salt possessed ν_{max} (Nujol) 1765 cm^{-1}. M.I.C. against E. coli NCTC 10418, 0.5μg/ml.

Example 23: 6β-[D,2-(2-[4-(3,5-Dihydroxybenzamido)phenyl]-pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

(a) 2-[4-(3,5-Dihydroxybenzamido)phenyl]-3-pyrazolin-5-one-4-carboxylic acid.

2-(4-Aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid (329mg, 1.5mmol) in dry dichloromethane (20ml) was stirred under nitrogen at room temperature and treated with triethylamine (630ul, 4.5mmol). The whole was stirred to solution and then treated with chlorotrimethylsilane (575ul, 4.5mmol) in dry dichloromethane (2ml), followed by stirring for 1 hour.

Simultaneously 3,5-diacetoxy benzoic acid, (357mg, 1.5mmol) was dissolved in dry dichloromethane (10ml), cooled to -20°C and treated sequentially with triethylamine (210ul, 1.5mmol) and thionyl chloride (122ul, 1.65mmol) in dry dichloromethane (1ml), followed by stirring for 0.75h at -20°C.

The per-silylated amino acid solution from above was cooled to -20°C and treated with the acid chloride solution, the whole was then allowed to warm to room temperature and stirred for 4 hours. Subsequently the reaction mixture was concentrated by evaporation and the residue partitioned between ethyl acetate and dilute sodium bicarbonate solution at pH 7.5. The aqueous layer was separated, acidified to pH 1.5 and extracted with ethyl acetate to give, after drying ($MgSO_4$) and evaporation, 190mg of crude product. This was then treated with dilute sodium hydroxide solution at pH 10 and stirred overnight. The solution was washed with ethyl acetate and acidified (5N HCl) to pH 1.5 to give, after ethyl acetate extraction, drying ($MgSO_4$), and evaporation, the title product (90mg), ν_{max} (Nujol) 1680, 1595, 1520, 1345, 1210 cm^{-1}, δ (D_6-Acetone + D_6-DMSO : D_2O) 6.42 (1H, t, J2Hz, 1 aryl proton), 6.78 (2H, d, J2Hz, 2 aryl protons), 8.42 (1H, s, C_3 pyrazole proton). Found: M^+, 311.0907; $C_{16}H_{12}N_3O_6$ requires M, 311.0906.

(b) 6-[D,2-(2-[4-(3,5-Dihydroxybenzamido)phenyl]-pyrazole-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt

2-[4-(3,5-Dihydroxybenzamido)phenyl-3-pyrazolin-5-one-4-

carboxylic acid, obtained in (a) above, (120mg, 0.34mmol) in dry dichloromethane (20ml) and methanol (2ml) was stirred at room temperature under nitrogen and treated with tri-n-octylamine (150µl, 0.34 mmol) to give a solution. The solvent was then removed by evaporation and the residue evacuated for a further 2h. The resultant octylamine salt was then suspended in dry dichloromethane (20ml) containing dry dimethylformamide (2 drops). The suspension was cooled to -20°C and treated with thionyl chloride (27.5µl, 0.37mmol) in dry dichloromethane (1ml). The whole was then stirred at -20°C for 10 mins and then treated with chlorotrimethylsilane (130µl , 1.02mmol) in dry dichloromethane (1ml), followed by triethylamine (143µl, 1.02mmol) to give a clear solution which was stirred 5 min at -10°.

Simultaneously ampicillin (119mg, 0.34mmol) in dry dichloromethane (20ml) was treated with triethylamine (96µl, 0.68mmol) and stirred to solution. This was then cooled to -20°C and treated with the silylated acid chloride solution from above, followed by stirring at room temperature for 2 hours. The reaction mixture was concentrated by evaporation and the residue partitioned between ethyl acetate and dilute sodium bicarbonate solution at pH 7.5. The aqueous layer was acidified to pH 1.5 (5NHCl) and then extracted with ethylacetate to give, after drying, ($MgSO_4$) and evaporation, 120 mg of crude product. The crude product was purified as the free acid by column chromatography (30mg) (SiO_2; 5:4:1 ethylacetate : isopropanol: water). The free acid possessed δ(D_6-Acetone + D_2O) 1.47, 1.56 (2 x 3H, 2s, gemdimethyl), 4.27 (1H, s, C_3 penicillin proton), 5.50 (2H, ABq, J4Hz, C_5 and C_6 penicillin proton), 5.88 (1H, s, -CHCON-), 6.45 (1H, t, J2Hz, 1 aryl proton, 6.84 (2H, d, J2Hz, 2 aryl protons), 7.15-7.92 (9H, m, 9 aryl protons), 8.43 (1H, s, C_3-pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17(b). The sodium salt possessed ν_{max} (Nujol) 1760 cm^{-1}. M.I.C. against E.coli NCTC 10418, 0.25µg/ml.

Example 24. 6β-[D,2-Phenyl-2-(2-[4-(2,4,6-triacetoxybenzamido) phenyl]-pyrazol-3-in-5-one-4-carbonylamino)]acetamido penicillanic acid, sodium salt.

(a) 2-(4-[2,4,6-Triacetoxybenzamido]phenyl)-3-pyrazolin-5-one-4-carboxylic acid.

2-(4-Aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid (219mg, 1mmol) in dry dichloromethane (20ml) was treated with triethylamine (420µl, 3mmol) and stirred at room temperature under nitrogen to solution. This was then treated with chlorotrimethylsilane (381µl, 3mmol) in dry dichloromethane (1ml) and stirred for 1 hour at room temperature.

Simultaneously 2,4,6 triacetoxybenzoic acid (296mg, 1mmol) in dry dichloromethane (20ml) was stirred at room temperature under nitrogen to solution. This was then cooled to -20°C, and treated with triethylamine (140µl, 1mmol) and thionyl chloride (81µl, 1.1mmol) in dry dichloromethane (1ml), followed by stirring for 10 mins at -20°C.

The persilylated amino acid solution from above was cooled to -20°C and treated with the benzoic acid chloride solution and the whole was then allowed to warm to room temperature and stirred for 2 hours. The reaction mixture was diluted with distilled tetrahydrofuran and the dichloromethane removed by evaporation. Water was then added and the pH adjusted to 2 (HCl). The whole was stirred for 20 mins. The tetrahydrofuran was then removed by evaporation and the aqueous suspension extracted into ethylacetate to give, after drying ($MgSO_4$) and evaporation, the title product (458mg). ν_{max} (Nujol) 1780, 1720, 1660, 1615, 1580, 1190 cm^{-1} δ(D_6-Acetone) 2.14 (6H, s, $-OCOCH_3$, o-to pyrazole), 2.25 (3H, s, para acetoxy), 6.90 (2H, s, benzamido aryl protons), 7.74 (4H, s, 4-aryl protons), 8. 40 (1H, s, C_3 pyrazole proton).

(b) 6β-[D,2-Phenyl-2-(2-[4-(2,4,6-triacetoxy benzamido)phenyl]-pyrazole-3-in-5-one-4-carbonylamino)]acetamido penicillanic acid, sodium salt.

The title compound was prepared from 2-(4-[2,4,6-triacetoxy-benzamido] phenyl)-3-pyrazolin-5-one-4-carboxylic acid, obtained in

(a) above, in a manner analogous to example 17 (b). The free acid possessed $\delta(D_6$-Acetone + $D_2O)$ 1.48, 1.58 (2 X 3H, 2s, gemdimethyl), 2.21 (6H, s, $-OCOCH_3$, o- to pyrazole), 2.30 (3H, s, para acetoxy), 4.30 (1H, s, C_3 penicillin proton), 5.55 (2H, ABq, J4Hz, C_5 and C_6 penicillin protons), 5.93 (1H, s, -CHCON-), 7.00 (2H, s, m-benzamido-protons), 7.15-7.65 (5H, m, ampicillin phenyl protons), 7.72 (4H, s, pyrazole phenyl protons), 8.50 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17 b. The sodium salt possessed ν_{max} (Nujol) 1765 cm^{-1}. M.I.C. against E. coli NCTC 10418, 2.0µg/ml.

Example 25 6β-[D,2-(2-[4-Formamido phenyl]-pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

a) 2-(4-Formamidophenyl)-3-pyrazolin-5-one-4-carboxylic acid.

2-(4-Aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid (438mg, 2mmol) in dry dichloromethane (40ml) was stirred at room temperature under nitrogen and treated sequentially with triethylamine (840µl, 6mmol) and chlorotrimethylsilane (780µl, 6mmol) in dry dichloromethane (1ml). After stirring for 1 hour the solution was treated with formic acetic anhydride (165ml, 2mmol) in dry dichloromethane (2ml) and the whole stirred overnight. The reaction mixture was then concentrated by evaporation and the residue partitioned between between ethylacetate and dilute sodium bicarbonate solution at pH 8.5. The aqueous layer was separated and acidified to pH 1.5 (5 NHCl) to give a precipitate of the title product which was filtered, washed with water and dried in vacuo (348mg), ν_{max} (Nujol) 1700, 1640, 1610, 1595, 840 cm^{-1}, $\delta(D_6\text{-DMSO} + CD_3OD)$ 7.65 (4H, s, aryl protons), 8.21 (1H, s), 8.52 (1H, s). Found: M^+; 247.0588; $C_{11}H_4N_3O_4$ requires M, 247.0593.

b) 6β-[D,2-(2-[4-Formamidophenyl]-pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

The acid as obtained in (a) above (124mg, 0.5mmol) was suspended in dry dichloromethane (20ml) containing dry dimethylformamide (2 drops) and treated with tri-n-octylamine (220µl, 0.5mmol). The suspension was stirred under nitrogen, cooled to -20°C and treated with thionyl chloride (42µl, 0.55mmol) in dry dichloromethane (2ml). After stirring for 20 mins at -20°C the reaction mixture was sequentially treated with chlorotrimethylsilane (64µl, 0.5mmol) in dry dichloromethane (1ml) and triethylamine (70µl, 0.5mmol), and stirred 5 minutes further at -10°C. Simultaneously ampicillin (175mg, 0.5mmol) in dry dichloromethane (10ml) was treated with triethylamine (140µl, 1mmol) and stirred at room temperature under nitrogen to solution. This was then cooled to -20°C and treated with the silylated pyrazole acid chloride solution from above. The whole was then allowed to warm to room temperature and stirred for 2 hours.

The reacti n mixture was concentrated by evaporation and the residue partitioned between dilute sodium bicarbonate solution at pH 7.5 and ethyl acetate. The aqueous layer was separated, acidified to pH 1.5 (5NHCl), and extracted with ethylacetate to give, after drying ($MgSO_4$) and evaporation, the crude title product (90mg). The crude product was purified as the free acid by column chromatography (20mg) (SiO_2, 5:4:1 ethylacetate: isopropanol:water). The free acid possessed δ(D_6-Acetone + D_2O) 1.50, 1.59 (2 x 3H, 2s, gemdimethyl), 4.32 (1H, s, C_3 penicillin proton), 5.75 (2H, ABq, J4Hz, C_5 and C_6 penicillin protons), 6.01 (1H, s, -CHCON-), 7.10-7.80 (9H, m, 9 aryl protons), 8.41 (1H, s), 8.61 (1H, s). The free acid was converted to the sodium salt in a manner analogous to example 17 (b). The sodium salt possessed ν_{max} (Nujol), 1760 cm^{-1}.

M.I.C. against E. coli NCTC 10418, 1.0µg/ml.

Example 26. 6β-[D,2-(4-Hydroxyphenyl)-2-(2-[4-Methoxycarbonyl-amino phenyl] pyrazol -3-in-5-one-4-carbonylamino)] acetamido penicillanic acid, sodium salt.

The title compound was prepared from 2-(4-methoxycarbonylamino-phenyl)-3-pyrazolin-5-one-4-carboxylic acid, obtained in example 17a in a manner analogous to example 7, except that it was found necessary to further purify the sodium salt by HP20SS chromatography (water → 1:1 acetone:water). The free acid possessed δ(D_6-acetone + D_2O) 1.48, 1.58 (2 x 3H, 2s, gemdimethyl), 3.70 (3H, s, $-O\underline{CH}_3$), 4.28 (1H, s, C_3 penicillin proton), 5.57 (2H, ABq, C_5 and C_6 penicillin protons), 5.79 (1H, s, -CHCON-), 6.78 (2H, d, J8Hz, protons o to OH), 7.32 (2H, d, J8Hz, protons m-OH), 7.58 (4H, s, pyrazole aryl protons), 8.42 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17 (b).
M.I.C. against E. coli 10418, 2.0μg/ml.

Example 27. 6β-[D,2-(2-[4-(4-Aminosulphonyl benzamido)phenyl] pyrazole-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

a) 2-[4-{4-Aminosulphonyl benzamido}phenyl]-3-pyrazolin-5-one-4-carboxylic acid.

4-Sulphonamidobenzoic acid (202mg, 1mmol) in dry distilled tetrahydrofuran (20ml) was stirred at room temperature under nitrogen to solution and then treated sequentially with triethylamine (140µl, 1mmol) and thionylchloride (81µl, 1.1mmol) in T.H.F. (1ml), followed by stirring for 4 hours.

Simultaneously 2-(4-aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid (219mg, 1mmol) in dry dichloromethane (20ml) was treated sequentially with triethylamine (420µl, 3mmol) and chlorotrimethyl silane (381µl, 3mmol) in dry dichloromethane (2ml), followed by stirring at room temperature under nitrogen for 1 hour.

The benzoic acid chloride from above was then treated with the persilylated amino acid solution and the whole stirred for 2 hours. The reaction mixture was concentrated by evaporation and the residue portioned between ethyl acetate and dilute sodium bicarbonate solution at pH 8.0. Subsequent separation of the aqueous layer followed by acidification to pH 1.5 (5 NHCl) produced a precipitate of the title product which was filtered, washed with water and dried in vacuo over phosphorous pentoxide (202 mg). ν_{max} (Nujol) 1650, 1605, 1580, 1530, 1510 cm^{-1}, δ(D_6-Acetone + D_6-DMSO + D_2O) 7.82 (4H, s, pyrazole aryl protons), 8.03 (4H, ABq, J9Hz, benzamido aryl protons), 8.60 (1H, s, C_3 pyrazole proton). Found: M^+, 358.0737; $C_{16}H_{14}N_4O_4S$ requires M, 358.0736.

b) 6β-[D,2-(2-[4-(4-Aminosulphonyl benzamido)phenyl] pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

The acid obtained in (a) above (90mg, 0.22mmol) was suspended in dry distilled tetrahydrofuran (20ml) containing dry dimethyl formamide(1 drop) and treated sequentially with triethylamine (33µl, 0.22 mmol) and thionyl chloride (18µl, 0.24mmol) in dry dichloromethane (1ml) followed by stirring under nitrogen at -20°C

for 20 mins. This was then treated sequentially with chlorotrimethyl silane (29µl, 0.22mmol) in dry dichloromethane (1ml) and triethylamine (33µl, 0.22mmol), and stirred 5 minutes. Simultaneously ampicillin (77mg, 0.22mmol) in dry dichloromethane (10ml) was treated at room temperature under nitrogen with triethylamine (66µl, 0.45mmol) and stirred to solution. This was then cooled to -20°C and treated with the silylated pyrazole acid chloride solution from above. The whole was allowed to warm to room temperature and stirred for 2 hours, followed by concentration of the reaction mixture by evaporation. The residue was partitioned between ethylacetate and dilute sodium bicarbonate solution at pH 8. Subsequent separation of the aqueous layer and acidification to pH 1.5 (5 NHCl) gave, after ethylacetate extraction, drying ($MgSO_4$) and evaporation, the crude title product (90mg). The crude product was purified as the free acid by column chromatography (SiO_2, 5:4:1 ethyl acetate : isopropanol : water). The free acid possessed δ(D_6-Acetone + D_2O) 1.47, 1.57 (2 x 3H, 2s, gemdimethyl), 4.28 (1H, s, C_3 penicillin proton), 5.55 (2H, ABq, J4Hz, C_5 and C_6 penicillin protons), 5.92 (1H, s, -CHCON-), 7.10-8.20 (13H, m, aryl protons), 8.51 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17(b). The sodium salt possessed ν_{max} (Nujol) 1760 cm^{-1}.

M.I.C. against E. coli. NCTC 10418, 1.0µg/ml.

Example 28. 6β-[D,2-(2-[4-(3,4-Dihydroxybenzamido)phenyl]pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

The penicillin sodium salt obtained as in example 8(b) (25mg, 0.03mmol) was dissolved in water (10ml) and the pH raised to 9 with sodium bicarbonate solution. The whole was stirred for 1.75h, and then acidified to pH 1.5 (5N HCl). Ethylacetate extraction produced after drying ($MgSO_4$) and evaporation, the title product (18mg). The free acid possessed δ(CD_3OD) 1.43, 1.53 (2 x 3H, 2s, gemdimethyl), 4.28 (1H, s, C_3 penicillin proton), 5.50 (2H, ABq, J4Hz, C_5 and C_6 penicillin protons), 5.80 (1H, s, -CHCON-), 6.81 (1H, d, J8Hz, 1 aryl proton), 7.38 (7H, m, 7 aryl protons), 7.67 (4H, s, 4 aryl protons), 8.36 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17 (b). M.I.C. against E. coli NCTC 10418, 0.06μg/ml.

Example 29. 6β-[D,2-(2-{3-Methoxycarbonylaminophenyl}pyrazole-3-in-5-one-4-carbonylamino)-2-phenyl]acetamido penicillanic acid, sodium salt.

a) 4-Ethoxycarbonyl-2-(3-nitrophenyl)-3-pyrazolin-5-one.

3-Nitrophenylhydrazine (1.54g, 10.1mmol) was dissolved in dry ethanol (50ml) and treated with 1N sodium ethoxide solution (22.2ml, 22.2mmol). This was then treated with diethylethoxymethylene malonate (2.18ml, 10.1mmol) and the whole stirred at room temperature under nitrogen for 3 hours. Water was then added and the pH lowered to 4.0 (5N HCl). The ethanol was removed by evaporation and the solid filtered, washed with water and dried in vacuo over phosphorous pentoxide, (2.73g), recrystallised from ethylacetate, mp. 175°C, ν_{max} (Nujol) 1680, 1605, 1540, 1530 cm^{-1}, δ(D_6- Acetone + D_6DMSO) 1.35 (3H, t, J7Hz, $OCH_2\underline{CH_3}$), 4.30 (2H, q, J7Hz, -$O\underline{CH_2}CH_3$), 7.60-8.78 (4H, m, 4 aryl protons), 8.88 (1H, s, C_3 pyrazole proton). Found: M^+, 277.0703; $C_{12}H_{11}N_3O_5$ requires M, 277.0699.

b) 2-(3-Aminophenyl)-4-ethoxycarbonyl-3-pyrazolin-5-one.

The nitro ester obtained in (a) above (277mg, 1mmol) was dissolved in distilled tetrahydrofuran (20ml) and treated with 10% palladium/charcoal catalyst (50mg). The whole was then hydrogenated at s.t.p. for 1 hour, at which point there was no nitro ester remaining. The catalyst was removed by filtration using celite and the celite washed well with distilled tetrahydrofuran. The filtrate was then evaporated to dryness to yield the crude title product as a foam (247mg). The crude product was purified by column chromatography (112mg) (SiO_2, 1:1 Hexane:Ethyl acetate), ν_{max} (CH_2Cl_2) 1710 sh, 1680, 1625, 1585, 1510 cm^{-1}, δ(D_6-Acetone) 1.31 (3H, t, J7Hz, -$OCH_2\underline{CH_3}$), 4.35 (2H, q, J7Hz, -$O\underline{CH_2}CH_3$), 5.65 (2H, bs, -NH_2 [exchangeable D_2O]), 6.50-7.40 (4H, m, aryl protons), 8.43 (1H, s, C_3 pyrazole proton).

c) 2-(3-Aminophenyl)-3-pyrazolin-5-one-4-carboxylic acid.

The amino ester obtained in (b) above (864mg, 3.49mmol) was suspended in 0.5N sodium hydroxide solution (18ml) and stirred on a boiling water bath under nitrogen for 1 hour. The resultant solution was then washed with ethylacetate, acidified to pH 2.5 (5NHCl), and extracted with a large volume of ethylacetate (750ml)

to give, after drying ($MgSO_4$) and evaporation, the title product (509mg) ν_{max} (Nujol) 1660, 1580 cm^{-1}, δ(D_6-DMSO + D_6 - Acetone) 6.40-7.40 (4H, m, 4 aryl protons), 8.47 (1H, s, C_3 pyrazole proton).

d) 2-(3-Methoxycarbonylaminophenyl)-3-pyrazolin-5-one-4-carboxylic acid.

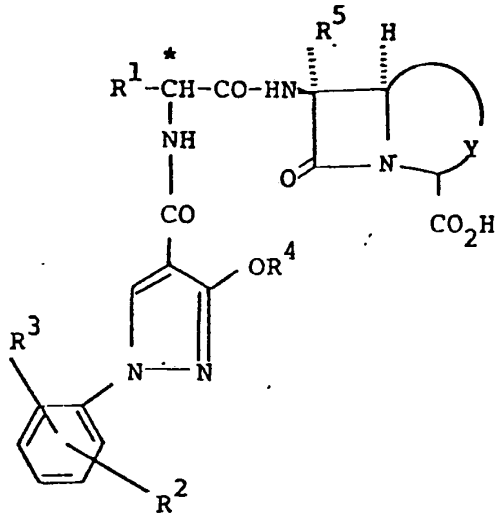
The amino acid obtained in (c) above, (219mg, 1mmol), was suspended in dry dichloromethane (20ml) and sequentially treated with triethylamine (420µl, 3mmol), and chlorotrimethylsilane (381µl, 3mmol), followed by stirring at room temperature under nitrogen for 1 hour. The solution was then treated with methylchloroformate (85µl, 1.1mmol) in dry dichloromethane (1ml) followed by stirring at room temperature for 4 hours. The reaction mixture was diluted with distilled tetrahydrofuran, concentrated by evaporation, and the residue partitioned between ethyl acetate and dilute sodium bicarbonate solution at pH 8. The aqueous layer was separated, acidified to pH 1.5 (5NHCl) and then extracted with ethylacetate, to give, after drying ($MgSO_4$) and evaporation, the title product (136mg) ν_{max} (Nujol) 1720 sh, 1700, 1610, 1585 cm^{-1}. δ(D_6- Acetone + CD_3OD) 3.79 (3H, s, $NHCOO\underline{CH_3}$), 7.45 (3H, m, 3 aryl protons), 8.02 (1H, m, 1 aryl proton), 8.51 (1H, s, C_3 pyrazole proton).

e) 6β-[D,2-(2-[3-Methoxycarbonylaminophenyl] pyrazol-3-in-5-one-4-carbonylamino)-2-phenyl] acetamido penicillanic acid, sodium salt.

The title compound was prepared from the acid obtained in (d) above in a manner analogous to example 17 (b). The free acid possessed δ(D_6-Acetone + D_2O) 1.37, 1.55 (2 x 3H, 2s, gemdimethyl), 3.72 (3H, s, $CO_2\underline{CH_3}$), 4.34 (1H, s, C_3 penicillin proton), 5.48 (1H, d, J4Hz, C_5 penicillin proton), 5.64 (1H, d, J4Hz, C_6 penicillin proton), 7.41 (8H, m, 8 aryl protons), 8.03 (1H, m, 1 aryl proton), 8.59 (1H, s, C_3 pyrazole proton). The free acid was converted to the sodium salt in a manner analogous to example 17 (b). The sodium salt possessed ν_{max} (Nujol) 1765 cm^{-1}.
M.I.C. against E. coli NCTC 10418, 2µg/ml.

Claims

1. A compound of the general formula (I) or a pharmaceutically acceptable salt or in-vivo hydrolysable ester thereof:



(I)

in which

R^1 denotes a phenyl group, a substituted phenyl group, or a 5- or 6-membered heterocyclic ring containing one, two, or three heteroatoms selected from oxygen, sulphur and nitrogen, and being unsubstitutd or substituted by one or more substituents selected from hydroxy, amino, halogen and (C_{1-6})alkoxy;

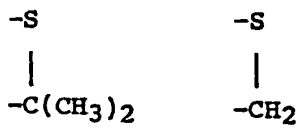
R^2 denotes a substituted amino group;

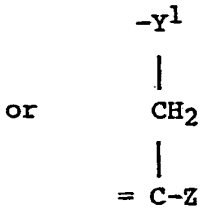
R^3 denotes hydrogen or a (C_{1-6})alkyl group;

R^4 denotes hydrogen, a methyl group, or an acetyl group;

R^5 denotes hydrogen, a methoxy group, or an -NHCHO group;

Y denotes:





Y^1 denotes an oxygen atom, a sulphur atom or a $-CH_2-$ group;

Z denotes hydrogen, a halogen atom or an organic group.

2. A compound as claimed in claim 1, wherein Y is $-S-C(CH_3)_2-$ or $-S-CH_2-C(CH_2-Q)=$.

3. A compound as claimed in claim 1, wherein R^5 denotes H or -NHCHO.

4. A compound as claimed in claim 1, wherein R^1 denotes phenyl or substituted phenyl.

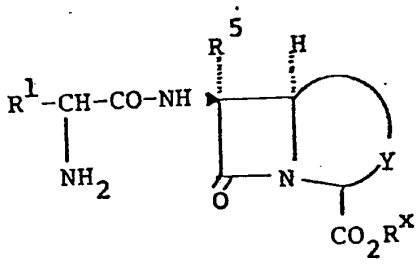
5. A compound as claimed in claim 1, wherein R^2 denotes -NHCOR, $-NHCO_2R$ or -NHCONHR, wherein R denotes hydrogen, unsubstituted or substituted hydrocarbon, or unsubstituted or substituted heterocyclyl.

6. A compound as claimed in claim 1, wherein R^3 denotes hydrogen.

7. A compound as claimed in claim 1, wherein R^4 denotes hydrogen.

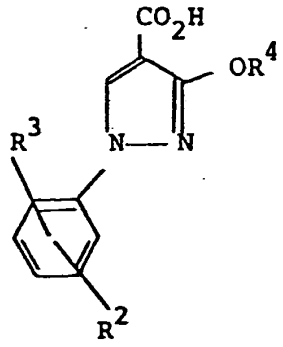
8. A process for the preparation of a compound of the general formula (I) defined in claim 1, or a pharmaceutically acceptable salt or in-vivo hydrolysable ester thereof, which process comprises:

(a) reacting a compound of formula (III):



(III)

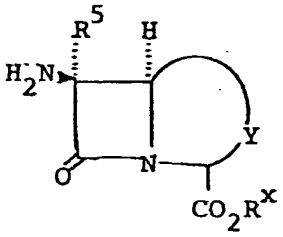
wherein the amino group is optionally substituted with a group which permits acylation to take place, R^1, R^5 and Y are as defined in claim 1, any reactive groups may be protected, and R^x is hydrogen or a carboxyl-blocking group, with an N-acylating derivative of a compound (IV):



(IV)

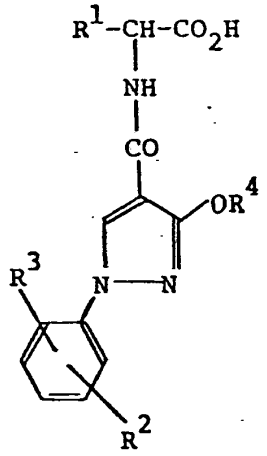
wherein R^2, R^3 and R^4 are as defined in claim 1, and wherein any reactive groups may be protected; or

(b) reacting a compound of formula (V):



(V)

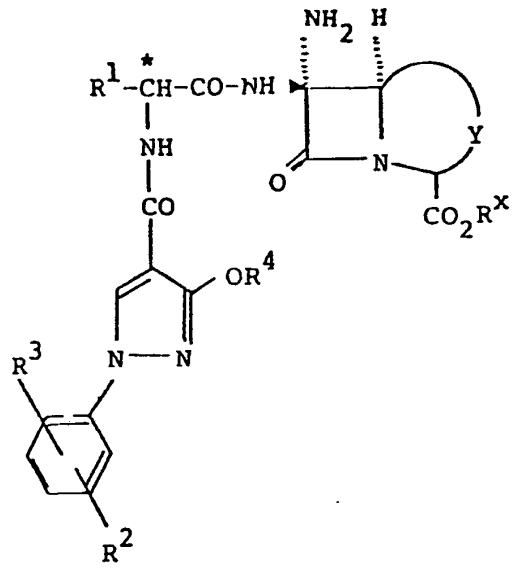
wherein the amino group is optionally substituted with a group which permits acylation to take place and R^5, R^x and Y are as defined in claim 1, with an N-acylating derivative of an acid of formula (VII):



(VII)

wherein R^1, R^2, R^3 and R^4 are as defined in claim 1 and any reactive groups therein may be protected; or

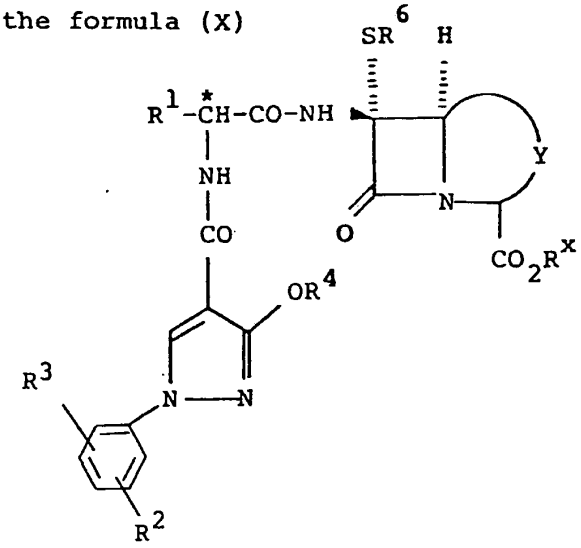
(c) when R^5 is -NHCHO, formylating a compound of formula (IX):



(IX)

wherein R^1, R^2, R^3, R^4, R^x and Y are defined in claim 1 and any reactive groups may be protected; or

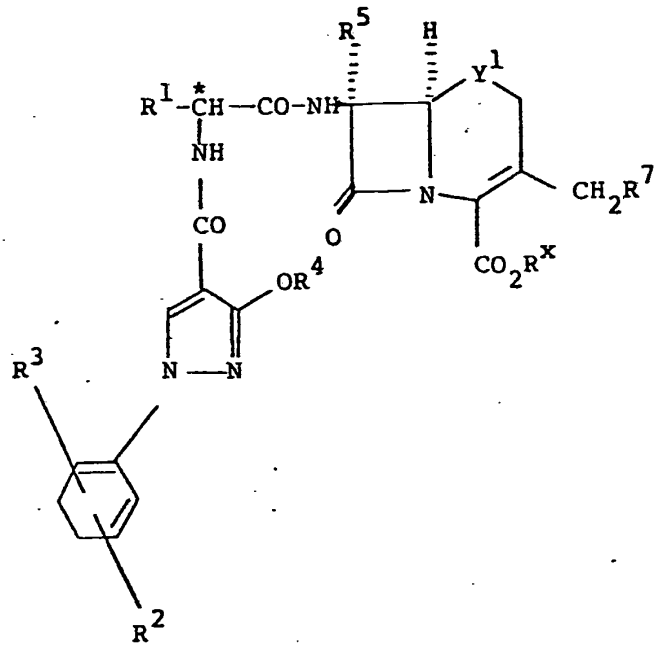
(d) when R^5 is methoxy, reacting a compound of the formula (X)



(X)

wherein R^1, R^2, R^3, R^4, R^x and Y are as defined in claim 1, and R^6 is (C_{1-6})alkyl, aryl or benzyl, with methanol in the presence of a metal ion; or

(e) when Y is $-Y^1-CH_2-C(-CH_2-S-Het)=$, wherein Y^1 is as defined in claim 1 and Het denotes a heterocyclyl group, reacting a compound of formula (XIV):



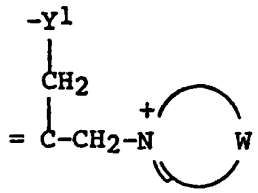
(XIV)

wherein R^1, R^2, R^3, R^4, R^5, R^x and Y^1 are as defined in claim 1, and R^7 is a leaving group, with the proviso that when R^7 is an acyloxy group, $-CO_2R^x$ is in free acid form or a salt thereof, and wherein any reactive groups may be protected, with a thiol of formula

HetSH

wherein Het is defined as above; or

(f) when Y is



wherein Y^1 is as defined in claim 1 and W represents the residue of a pyridinium group unsubstituted or substituted by one or two groups selected from (C_{1-6})alkyl, (C_{1-6})alkoxy, hydroxyalkyl, (C_{1-6})alkenyl, alkoxyalkyl, carboxyalkyl, sulphonylalkyl, carbamoylmethyl, carbamoyl, trifluoromethyl, hydroxy, halogen, oxo, and aminoalkyl; reacting a compound of formula (XIV) as defined in (e) above with an appropriately substituted pyridine; and

after any of steps (a) to (f) above, if necessary, carrying out one or more of the following steps:

(i) removing any carboxyl-blocking group R^X;

(ii) removing any protecting groups on the side-chain group;

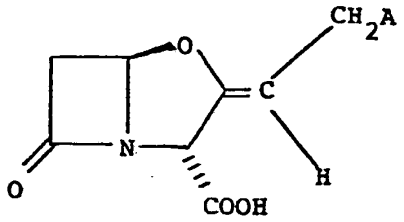
(iii) converting one group Z to a different group Z; and/or

(iv) converting the product into a salt or in-vivo hydrolysable ester.

9. A pharmaceutical composition comprising a compound of formula (I) as defined in claim 1, together with a pharmaceutical carrier or excipient.

10. A composition as claimed in claim 9, which additionally comprises:

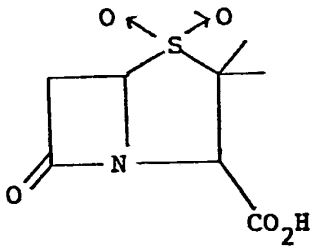
(a) a compound of formula (XVI) or a pharmaceutically acceptable salt or ester thereof:



(XVI)

wherein A is hydroxyl, substituted hydroxyl, thiol, substituted thiol, amino, (mono or di)-hydrocarbyl-substituted amino, or (mono or di)-acylamino; and/or

(b) a compound of formula (XVII) or a pharmaceutically acceptable salt or in-vivo hydrolysable ester thereof:



(XVII)

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00161

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 07 D 499/68; C 07 D 499/00; C 07 D 501/20; C 07 D 498/04; C 07 D 471/04; A 61 K 31/43; // C 07 D 231/20; ./.

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 07 D 499/00; C 07 D 501/00; C 07 D 498/00; C 07 D 471/00; A 61 K 31/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	EP, A, 0090656 (BEECHAM) 5 October 1983, see page 42, example 7; pages 74-5, example 24 and claims (cited in the application) --	1-4,8,9
Y	US, A, 4315933 (T.F. MICH) 16 February 1982, see claims --	1,9
A	Chemical Abstract, volume 86, nr. 9, 28 February 1977 (Columbus, Ohio, US) see page 463, abstract 55670z & JP, A, 7686464 (KOHJIN CO. LTD.) 29 July 1976	1

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
2nd July 1985	02 AOUT 1985
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	G.L.M. Kruydenberg

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00161

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: (C 07 D 498/04, 265/00, 205/00) (C 07 D 471/04,221/00,205/00)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	G.L.M. Kruydenberg

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 85/00161 (SA 9369)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/07/85

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

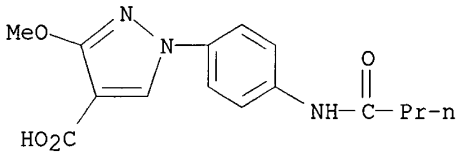
Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0090656	05/10/83	JP-A- 58183692	26/10/83
US-A- 4315933	16/02/82	None	

L31 ANSWER 97 OF 136 CAPLUS COPYRIGHT 2002 ACS
AN 1986:626174 CAPLUS
DN 105:226174
TI .beta.-Lactam derivatives, and compositions containing them
IN Taylor, Andrew William; Cook, Richard Thomas
PA Beecham Group PLC, UK
SO PCT Int. Appl., 99 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

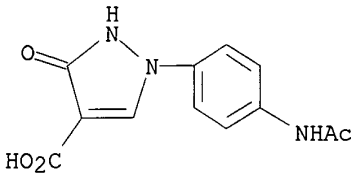
PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 8504878	A1	19851107	WO 1985-GB161	19850412
W: GB, JP, US				
RW: CH, DE, FR, GB, IT, NL				
EP 177596	A1	19860416	EP 1985-902070	19850412
R: CH, DE, FR, GB, IT, LI, NL				
PRAI GB 1984-9986		19840417		

AB .beta.-Lactams I [R1 = (un)substituted Ph; OH, NH2, halo, or C1-6 alkoxy (un)substituted 5- or 6-membered heterocyclyl with 1-3 hetero atoms (O, S, or N); R2 = substituted NH2; R3 = H, C1-6 alkyl; R4 = H, Me, Ac, R5 = H, MeO, NHCHO; Y = SCMe2, SCH2, Y1CH2C(Z):; Y1 = O, S, CH2; Z = H, halo, org. group], useful as antibacterials, were prepd. 2-p-Aminophenyl-4-ethoxycarbonyl-3-pyrazolin-5-one was sapond. with boiling aq. 0.5 N NaOH to give 97% of the acid which was refluxed with (Me3Si)2NH and the product acetylated in CH2Cl2 to give 67% 2-p-acetylaminophenyl-3-pyrazolin-5-one-4-carboxylic acid. This was converted to the acid chloride which reacted with ampicillin to give 6.beta.-[D,2-(2-p-acetylaminophenyl-3-pyrazolini-5-one-4-carbonylamino)-2-phenyl]acetamidopenicillanic acid (II). II Na salt has a min. inhibitory concn. of 2.5 .mu.g/mL against Escherichia coli NCTC 10418.

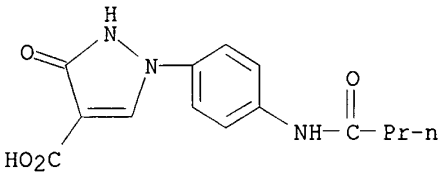
IT **105432-79-9P**
RL: SPN (Synthetic preparation); PREP (Preparation)
(prepn. and conversion of, to acid chlforide)
RN 105432-79-9 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 3-methoxy-1-[4-[(1-oxobutyl)amino]phenyl]- (9CI) (CA INDEX NAME)



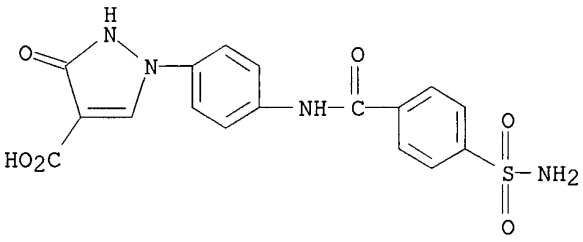
IT **105432-71-1P 105432-87-9P 105432-93-7P**
105433-12-3P 105433-17-8P
RL: SPN (Synthetic preparation); PREP (Preparation)
(prepn. and conversion of, to acid chloride)
RN 105432-71-1 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 1-[4-(acetylamino)phenyl]-2,3-dihydro-3-oxo- (9CI) (CA INDEX NAME)



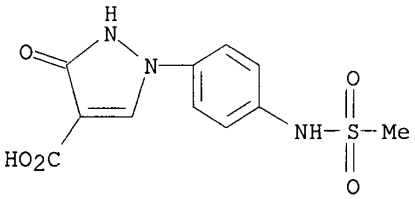
RN 105432-87-9 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 2,3-dihydro-3-oxo-1-[4-[(1-oxobutyl)amino]phenyl]- (9CI) (CA INDEX NAME)



RN 105432-93-7 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 1-[4-[[4-(aminosulfonyl)benzoyl]amino]phenyl]-2,3-dihydro-3-oxo- (9CI) (CA INDEX NAME)

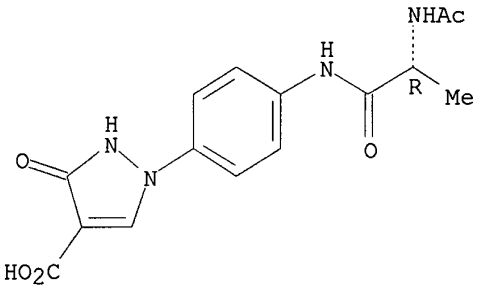


RN 105433-12-3 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 2,3-dihydro-1-[4-[(methylsulfonyl)amino]phenyl]-3-oxo- (9CI) (CA INDEX NAME)



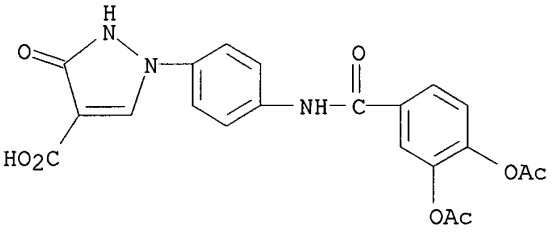
RN 105433-17-8 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 1-[4-[[2-(acetylamino)-1-oxopropyl]amino]phenyl]-2,3-dihydro-3-oxo-, (R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

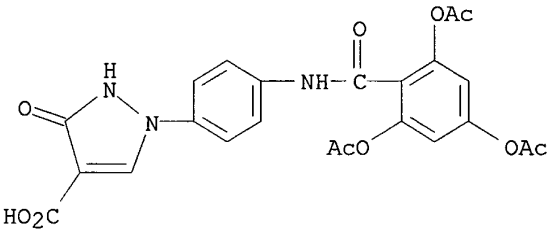


IT **105432-90-4P 105433-03-2P 105433-21-4P**
105433-27-0P 105433-30-5P 105433-45-2P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation)
(prepn. and reaction of)

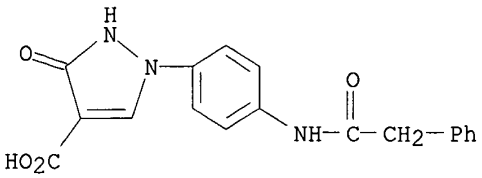
RN 105432-90-4 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 1-[4-[[3,4-bis(acetyloxy)benzoyl]amino]phenyl]-2,3-dihydro-3-oxo- (9CI) (CA INDEX NAME)



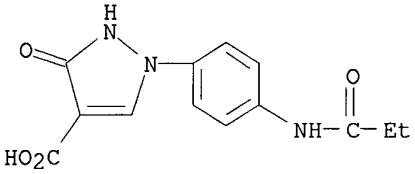
RN 105433-03-2 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 2,3-dihydro-3-oxo-1-[4-[[2,4,6-tris(acetyloxy)benzoyl]amino]phenyl]- (9CI) (CA INDEX NAME)



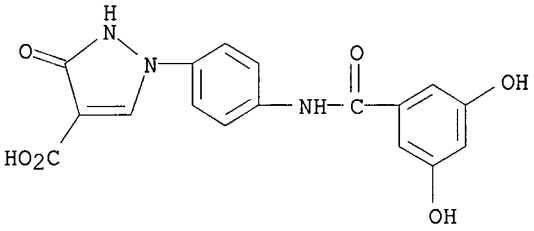
RN 105433-21-4 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 2,3-dihydro-3-oxo-1-[4-[(phenylacetyl)amino]phenyl]- (9CI) (CA INDEX NAME)



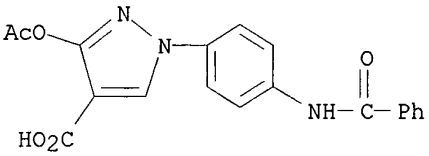
RN 105433-27-0 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 2,3-dihydro-3-oxo-1-[4-[(1-oxopropyl)amino]phenyl]- (9CI) (CA INDEX NAME)



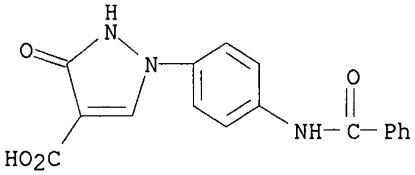
RN 105433-30-5 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 1-[4-[(3,5-dihydroxybenzoyl)amino]phenyl]-2,3-dihydro-3-oxo- (9CI) (CA INDEX NAME)



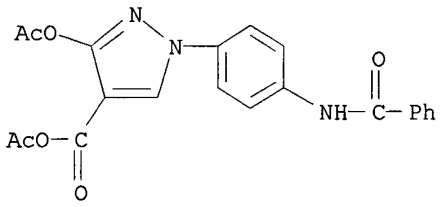
RN 105433-45-2 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 3-(acetyloxy)-1-[4-(benzoylamino)phenyl]- (9CI) (CA INDEX NAME)



IT **105432-82-4P**
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation)
(prepn. and reactions of)
RN 105432-82-4 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 1-[4-(benzoylamino)phenyl]-2,3-dihydro-3-oxo- (9CI) (CA INDEX NAME)



IT **105433-44-1P**
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation)
(prepn. and sapon. of)
RN 105433-44-1 CAPLUS
CN 1H-Pyrazole-4-carboxylic acid, 3-(acetyloxy)-1-[4-(benzoylamino)phenyl]-, anhydride with acetic acid (9CI) (CA INDEX NAME)



IT **105458-13-7P**
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation)
(prepn. and silylation of)
RN 105458-13-7 CAPLUS
CN 1H-Pyrazole-4-carbonyl chloride, 1-[4-(benzoylamino)phenyl]-2,3-dihydro-3-oxo- (9CI) (CA INDEX NAME)